A STUDY OF ANDROGEN CONJUGATES AND APOCRINE DIFFERENTIATION IN THE HUMAN BREAST

John Michael Dixon

BSc Hons (Med Sci) 1975

MB ChB (Edin) 1978

Thesis submitted for the Degree of Doctor of Medicine at Edinburgh University, 1984.

INDEX

INDEX (contd)

DECLARATION

This thesis has been composed by myself. The work presented is my own or that performed in cooperation with other members of a research group.

DEDICATION

I dedicate this thesis to my late father, John Michael Dixon, for his continuous encouragement and support during my school and undergraduate education.

ACKNOWLEDGEMENTS

I should like to express my gratitude to Dr W R Miller for supervision of this project and thesis, his enthusiasm and interest in the work and also for his friendship. I am indebted to Professor A P M Forrest for his support and encouragement and for allowing me to work in his Department.

In addition, I should like to thank the following individuals for direct assistance with this project and thesis: Mr W N Scott for teaching me the DHA sulphate radioimmunoassay and for performing the electrolyte analysis of cyst fluids; Dr T J Anderson for providing facilities and assistance with histological and immunohistochemical techniques used in this work; Dr R A Hawkins for organising the storage of cyst fluids, and the data on oestrogen receptors; Dr P L Yap for his data on proteins and immunoglobulins in cyst fluid; Mrs Sandy McLeod and Mrs Julie Baxter for collecting breast secretions; Miss June Telford for expert technical assistance; Mrs Anne McNeill for drawing the figures and the Department of Medical Illustration for reproducing them; Mrs Janet Wake for assistance in obtaining finance for this project and thesis and Dr R A Elton for statistical advice.

I am also grateful to Mrs Ann Dunsire for her swift and accurate typing of this thesis.

I should also like to express my thanks to the Wellcome Trust for the award of a Wellcome Surgical Research Fellowship, enabling me to perform this work and for financial support to attend scientific meetings in Salzburg and Amsterdam.

Finally, I would like to thank all the patients involved in this study, without whose help and cooperation this work would not have been possible.

ABSTRACT

The concentrations of the androgen conjugate DHA sulphate in human breast fluids and breast tissue have been studied. It has been shown that DHA sulphate is present within breast secretions, breast cyst fluids and breast tissues in much higher concentrations than those found in plasma.

The levels of DHA sulphate in breast cyst fluid have been shown to correlate directly with the K+ content and inversely with the Na+ content of the fluid. On the basis of the concentrations of K+, Na+ and DHA sulphate, it was possible to define two populations of breast cyst fluids and evidence has been presented to show that these are derived from different types of epithelium. One group of cysts are lined by apocrine epithelium and contain high concentrations of K+ and DHA sulphate and low concentrations of Na+. The other group are lined by flattened epithelium and have the reverse composition (low [K+], low [DHA sulphate], high [Na+]). Other components of cyst fluid also correlate directly with the nature of the epithelial lining.

The natural history of cystic disease has been shown to be closely related to cyst type and therefore to the composition of cyst fluid. Patients with apocrine cysts appear more likely to have multiple simultaneous cysts and more commonly develop sequential cysts. These patients are also more likely to have within their breasts lesions which are known to be risk factors for subsequent breast

cancer. Furthermore, a study of a group of patients with breast cancer showed a significantly greater number had a history of apocrine rather than flattened cysts. These findings suggest that patients with apocrine cysts may be at a relatively increased risk of subsequent breast cancer.

A study of the precursors of palpable breast cysts, microcysts, identified only a single population. Microcysts contain massive amounts of DHA sulphate, high levels of K+ and appear to be lined by epithelium with marked apocrine features.

Using an immunoperoxidase technique DHA sulphate was visualised in human breast secretions, apocrine epithelium and a proportion of human breast cancers. The amount of DHA sulphate present in carcinomas was greatest in these tumours which had a marked degree of apocrine differentiation.

These observations suggest that DHA sulphate is a marker of apocrine activity. Its presence within the breast is in keeping with the breast being a modified apocrine gland. Measurement of DHA sulphate within the breast may therefore give useful information on the degree of apocrine activity of the breast. Measurement of this hormone in breast cyst fluid appears to be particularly useful, as it gives information both on the likely natural history of the disease and appears to identify those patients at increased risk of epithelial proliferation.

ABBREVIATIONS USED IN THE TEXT

DHA	dehydroepiandrosterone
DHA-S	dehydroepiandrosterone sulphate
LH	luteinizing hormone
FSH	follicle stimulating hormone
Na+	sodium
K+	potassium
DAB	diaminobenzidine
Ig	immunoglobulin
S	sedimentation coefficient in Svedbergs
cpm	counts per minute
v/v	volume by volume

REFERENCES

et al used where more than one author

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The mammary gland is a complex endocrine target organ, its growth and secretory differentiation depending on an interplay of pituitary, ovarian, thyroid, adrenal and pancreatic hormones (Robyn 1983).

Growth is largely under the influence of oestrogens and progesterone. Oestrogens alone can promote the development of breast ducts and in synergism with progesterone, promote growth of lobules (Van Bogaert 1976, Robyn 1983). Differentiation of the breast epithelium is also influenced by oestrogens whilst progesterone has an effect on interlobular ducts (Bolander et al 1980). During pregnancy a combination of oestrogens, progesterone and prolactin stimulate growth of the terminal duct-lobular units. The presence of high concentrations of sex steroids and human placental lactogen inhibit lactogenesis in pregnancy but, after delivery, the fall in sex hormones results in lactogenesis due to the unopposed action of prolactin (Robyn 1983). The whole of this process of growth and differentiation is, however, only achieved in the presence of insulin, cortisol and thyroxine (Cowie et al 1980).

Hormones are thus the major factors in the development and growth of the normal breast. It thus follows that abnormalities of growth may reflect abnormalities in hormone levels. There is strong epidemiological evidence to suggest that hormones have an aetiological role in the development of benign and malignant breast

disease (A Miller et al 1980). Both diseases are more prominent in females than males. The incidence of cystic disease, the commonest benign condition of the breast, varies during reproductive life occurring in late premenopausal and perimenopausal women with symptoms disappearing after the menopause (Haagensen 1971, Marchesoni et al 1983). With regard to breast cancer, oophorectomy before the age of 35 markedly reduces risk, whereas risk is increased by a late natural menopause (MacMahon et al 1973). A link also exists between breast cancer and parity, age at first pregnancy and anovulatory cycles (Shapiro et al 1968, MacMahon et al 1973, Sherman et al 1974, Mauvais-Jarvis et al 1975, Korenman 1980a, Korenman 1980b, Kalache et al 1982).

Despite this evidence, studies of hormones in both plasma and urine of women with benign and malignant breast disease have yielded conflicting results. However, there are two important findings which are worthy of mention.

The first relates androgens and breast cancer - Bulbrook and Hayward (1967) collected urine from 4850 normal women on the island of Guernsey and later analysed the urine of women who developed cancer along with carefully matched controls. They showed that those women who developed breast cancer had significantly lower concentrations of aetiocholanolone in their urine than controls. This finding is particularly important as the abnormality in the urine was present before the disease was detected clinically. Aetiocholanolone in the urine is largely derived from plasma DHA sulphate and other related

C19 steroids. Studies of DHA and DHA sulphate in plasma have also indicated that patients at risk of breast cancer may have lower levels than controls (Wang et al 1975, Wang et al 1979), although this remains disputed (Chetty et al 1983). The role of these androgens in breast disease may be indirect. In postmenopausal women adrenal androgens provide the major precursors of oestrogens with conversion occurring peripherally (MacDonald et al 1967). It is of note that adipose tissue and breast tumours can also perform this conversion (Abul-Hajj et al 1975, Adams et al 1975, Miller et al 1976, Varella et al 1978).

The second series of observations relate to oestrogens and progesterone. There is evidence that anovulatory cycles, which occur at the extremes of reproductive life, may be related to benign and malignant breast disease (Mauvais-Jarvis 1979, Korenman 1980b, Berta et al 1983, Kuttenn et al 1983). In these situations, oestrogenic stimulation of target tissues occurs without the opposing action of progesterone. Support for this also comes from the finding that there is an inverse correlation between plasma progesterone and breast cancer risk (Bulbrook et al 1978). However, it should be noted that not all women with breast cancer have anovulatory cycles and not all women with anovulatory cycles develop benign or malignant breast disease (Korenman 1980b).

Although both these studies are convincing as individual investigations, these have not been confirmed by others and thus it is yet to be proven that abnormalities in plasma hormones are commonly associated with benign and malignant breast disease.

One possible explanation for the failure to identify consistent differences is that hormone levels in plasma and urine may not accurately reflect concentrations within the breast. There is difficulty, however, in obtaining information on tissue levels of hormones in the normal and diseased breast. Certain fluids can be obtained from the breast and include, milk during lactation, breast secretions collected from non-pregnant women by nipple aspiration (Sartorius 1973) and cyst fluid obtained by needle aspiration from patients with cystic disease.

Milk has a constant composition, is produced only in pregnancy or in association with hyperprolactinaemia and does not reflect the hormonal environment of the resting breast (Kulski et al 1977, Yap et al 1980).

In contrast, breast secretions and cyst fluid are more widely available and, as they are derived from the resting breast, may provide more representative information on breast hormone levels, at times when breast disease is being induced. Results from analysis of these two fluids have shown concentrations of oestrogens and androgens which are greatly in excess of those in plasma (Miller et al 1977, Raju et al 1977, Wynder et al 1977, Bradlow et al 1979,

Miller et al 1980, Bradlow et al 1981a, Miller et al 1981, Miller et al 1982, Bradlow et al 1983a, Bocuzzi et al 1983, Miller et al 1983). The androgen conjugate DHA sulphate, has been found in breast secretions at concentrations from 7 to 400 times greater than those in plasma and in cyst fluids at up to 100 times greater concentrations (Miller et al 1980, Miller et al 1981, Miller et al 1982, Miller et al 1983). It thus appears that hormone levels within plasma may not reflect those in the breast. At present, however, neither the origin nor the factors that affect the concentration of these hormones and their conjugates in breast derived fluids have been defined.

The aims of the present study were thus:

(1) to define further the composition of breast secretions and breast cyst fluids
(2) to investigate the origin of these fluids
(3) to determine factors which affect their composition
(4) to correlate composition of cyst fluids to the course of the disease and the associated risk of breast cancer
(5) to investigate the concentration of DHA sulphate in breast tissue and breast carcinomas and determine what factors influence these levels.

GENERAL MATERIALS AND METHODS

Materials

All chemicals, with the exception of those mentioned below, were of Analar grade and obtained from BDH Chemicals, Poole, Dorset.

1,2,6,7 ^{3}H DHA 14.5 Ci/mmol	The Radiochemical Centre, Amersham.
DHA DHA sulphate activated charcoal DAB alpha naphol phosphoric acid pararosaniline	Sigma Chemicals, St Louis, USA
Toluene scintillation grade	Fisons, Loughborough, England
Sephadex LH 20	Pharmacia Fine Chemicals, Uppsala, Sweden
Scintol 7	Koch-Light Labs, Colinbrock, England

Hydrogen Peroxide	May and Baker
Normal rabbit serum	Scottish Antibody Production Unit, Edinburgh
Haematoxylin	
Eosin	
Basic Fuchsin	Histolab, London.
Permount	
Diastase	
Glutaraldehyde	
Osmium tetraoxide	Emscope, Kent.
Epoxypropane	
Araldite	

Buffers

DHA sulphate assay buffer

2g gelatin, 2g sodium azide, 18g sodium chloride, 21.8g disodium hydrogen orthophosphate dihydrate and 12.4g sodium dihydrogen orthophosphate were dissolved in 2 litres of distilled water. Final pH 7.0.

Phosphate buffered saline (PBS)

0.6 g sodium dihydrogen orthophosphate, 5.7 g disodium hydrogen orthophosphate and 17.0g NaCl were dissolved in 1 litre of distilled water. Final pH 7.6.

Tris HC1 buffer

38.5 ml 0.1M HC1, 50 ml 0.1M Tris (12.114g/l) and 11.5ml distilled water pH 7.6.

Wolpoles buffer

155ml of 0.1M sodium acetate and 45ml of 0.1N acetic acid. Final pH 5.0.

Cacodylate buffer

0.1M sodium cacodylate was dissolved in distilled water made to pH 7.0 by addition of IN HCl.

Other solutions

Hexazotised pararosaniline

Solution A : pararosaniline 1g
2N HCl 25ml
Heat gently, cool and filter

Solution B : sodium citrate 4g
distilled water 100ml

before use equal parts of solutions A and B were added.

Dextran Coated Charcoal

0.625g% activated charcoal suspended in DHA sulphate assay buffer containing 0.0625g% Dextran T 70.

Scintillation fluid

200ml Scintol 7 and 4800ml Toluene.

Schiff's reagent

1g of basic fuchsin was dissolved in 200ml of boiling water and allowed to cool to 50°C before adding 2g of potassium metabisulphite. The mixture was allowed to cool before adding 2ml of concentrated hydrochloric acid and 2g of activated charcoal. This was left overnight in the dark at room temperature. The mixture was then filtered and ready for use.

Antibodies

The anti DHA antibody was kindly donated by Mr B Morris of the Department of Biochemistry, University of Surrey. This antibody was raised in sheep* and its specificity has been previously defined (Table I). Peroxidase conjugated rabbit immunoglobulins to sheep immunoglobulins was obtained from DAKO immunoglobulins, Denmark.

*Immunogen-DHA-3β monohemisuccinate conjugated to ovalbumin.

Dehydroepiandrosterone sulphate	100%
Dehydroepiandrosterone	95%
Dehydroepiandrosterone glucuronide	70%
Epiandrosterone sulphate	70%
Epiandrosterone	9.5%
Δ 4-androste ne-3,17 dione	4.0%
Androsterone sulphate	2.5%
Androsterone	2.0%
Aetiocholanolone sulphate	0.15%
5α Dihydrotestosterone	0.01%
Oestrone	0.01%
Testosterone	<0.01%
Testosterone sulphate	
Cholesterol	
Cholesterol sulphate	
3β-hydroxy-5-pregnen-20-one	

All values are the mean of three determinations.

Cross reaction calculated from ratio of molar mass of DHA sulphate to cross-reacting steroid, required to displace 50% of the radioligand, multiplied by 100.

Table I Cross-reactivity of various steroids with the anti DHA antibody (from Miller et al 1980)

Method of obtaining breast secretions by nipple aspiration

Breast secretions were obtained by one of two research nurses in the Department of Clinical Surgery by a standard technique. This technique which utilises the Sartorius suction cup as shown in Figure 1 is demonstrated in Figures 2 to 5. The nipple was first carefully cleaned with alcohol. Following this, the breasts were massaged, starting at the periphery of the breast and moving centrally towards the nipple. The suction cup was then placed over the nipple and negative pressure applied using a 20 ml syringe. Following this, if the woman produced secretions, these could be seen on the surface of the nipple and collected in a capillary tube of known diameter. All samples were then stored at -20°C prior to analysis.

Purification of ^{3}H DHA

^{3}H DHA was purified by chromatography on Sephadex LH20 prior to use. Between 20 and 40 μ Ci of ^{3}H steroid was dissolved in benzene:ethanol (9:1 v/v) and placed on a Sephadex LH column (15 x 1 cm). Elution was carried out with benzene:ethanol (9:1 v/v). One ml fractions were collected and the radioactivity monitored in each by counting 10 μl aliquots. The fractions with peak radioactivity were bulked together, dried down at 60°C under air and dissolved in 0.2 ml of ethanol. These stock solutions were then stored at 4°C

Figure 1 Sartorius nipple aspiration cup

Figure 2

Figure 3

Figure 4

Figures 2-4 Method of obtaining breast secretions using the Sartorius nipple aspiration cup

and diluted when required with assay buffer to form an appropriate working solution on the day of the assay.

Radioimmunoassay for DHA sulphate

This was performed using the method of Buster and Abrahams (1973). Samples of plasma, breast cyst fluid and breast secretions were diluted from between 50 and 1,000,000 times (v/v) with assay buffer and 0.5 ml assayed in duplicate as follows:

The samples were cooled to 4°C and incubated at that temperature overnight with 0.1 ml anti DHA antibody (diluted 1 in 27,500 v/v with assay buffer) and 0.1 ml [1,2,6,7 ^{3}H] DHA (containing approximately 10,000 cpm in assay buffer). Bound steroid was then separated from free by the addition of 0.2 ml of dextran coated charcoal at 0°C. After 20 minutes the samples were centrifuged at 2500 rpm for 10 minutes at 4°C. The supernatant containing the bound fraction was decanted into glass scintillation vials containing 10 ml of scintill ant, mixed and incubated for 2 hours at 37°C. The vials were then cooled to 4°C and counted x 2 for 10 minutes using a Packard Tri Carb Liquid Scintillation Spectrometer.

With each assay, known amounts of unlabelled DHA sulphate (0.025 - 5 ng/0.5 ml assay buffer) were taken through the assay so that a standard curve (cpm v log ng/0.5 ml DHA sulphate) could be plotted. The values for unknown samples were then calculated by determining the DHA sulphate in each diluted sample from the standard curve and

multiplying by the diluting factor. Standard plasmas, cyst fluids and breast secretions were also taken through each assay so that interassay variation could be corrected for. Having determined the value in ng/ml, these values were then converted to μ mol/l.
Because the antibody used in the assay cannot distinguish between DHA sulphate (Table I) and DHA, the result obtained represents the sum of both. However in all samples studied the amount of DHA sulphate is many hundred times greater than that of DHA and therefore the value obtained essentially reflects DHA sulphate concentrations (Miller et al 1980, Mason 1981).

Estimation of Na+ and K+ concentrations in breast fluid

This was performed by flame photometry on a 1 in 200 or a 1 in 400 dilution of breast cyst fluid or breast secretion in distilled water using an EEL model 150 flame photometer. Standard solutions containing known concentrations of Na+ and K+ were used on each occasion, to calibrate the machine and to check its accuracy during a series of measurements.

DHA sulphate localisation by Immunoperoxidase

Attempts were made to localise DHA sulphate in (i) formalin fixed tissue embedded in paraffin; (ii) frozen sections of formalin fixed material; and (iii) frozen sections of fresh tissue. Only on frozen sections of fresh tissue was positive staining obtained. This is

probaby due to DHA sulphate being extractable into both water and alcohol.

Frozen sections cut at between 3 and 5 μm were washed in PBS for 1 minute. The tissues were then incubated for 30 minutes in 10% normal rabbit serum (NRS). The slides were drained of excess serum and wiped. The sections were then incubated for 30 minutes with a 1 in 50 dilution of sheep antiserum to DHA diluted in 10% NRS and 5% normal human serum from a patient taking aminoglutethimide and whose plasma was known to contain almost undetectable levels of DHA sulphate. As a control, serial sections were incubated with a 1 in 10 dilution of normal sheep serum diluted in 10% NRS. After rinsing with the changes of PBS for 10 minutes, peroxidase conjugated rabbit anti sheep antibody diluted 1 in 50 in 10% NRS and 5% human serum was added for 30 minutes. The sections were rinsed x 3 for 10 minutes in PBS then a solution of DAB, 5 mg in 10 ml of Tris HCl buffer with 75 μl of hydrogen peroxide was applied for 5 minutes. The sections were then washed in water, counterstained with haematoxylin, dehydrated in graded alcohols and xylene and mounted with Permount.

A section of axillary skin containing apocrine glands and sections of human adrenal gland were used as positive controls (Figures 6,7). Human ovarian stroma was used as a negative control (Figure 8). Primary antisera absorbed with DHA sulphate was also substituted as a second negatively staining control as a test for the specificity of the antibody staining reaction.

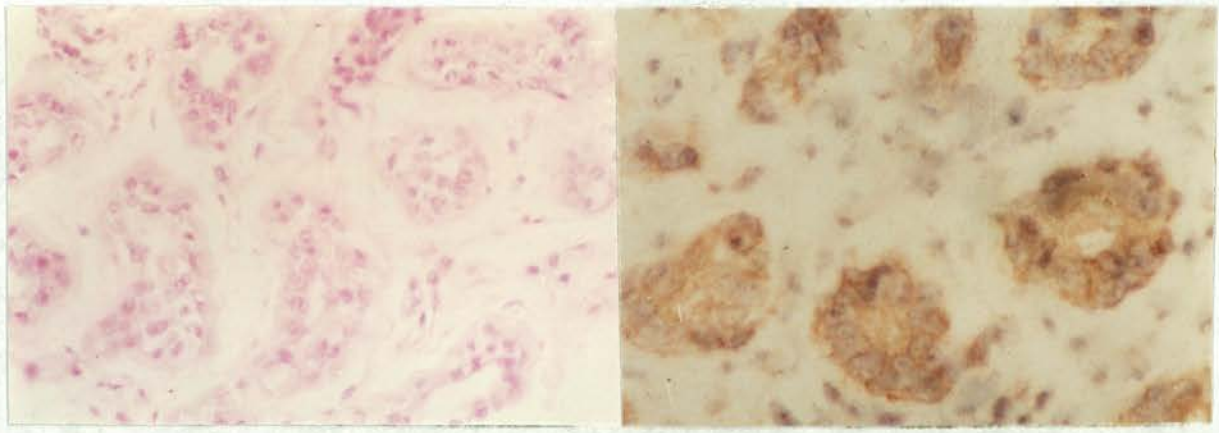


Figure 6 Human apocrine glands from axillary skin stained with the immunoperoxidase technique to show the presence of DHA sulphate - positive control - (H & E and frozen section x 200)

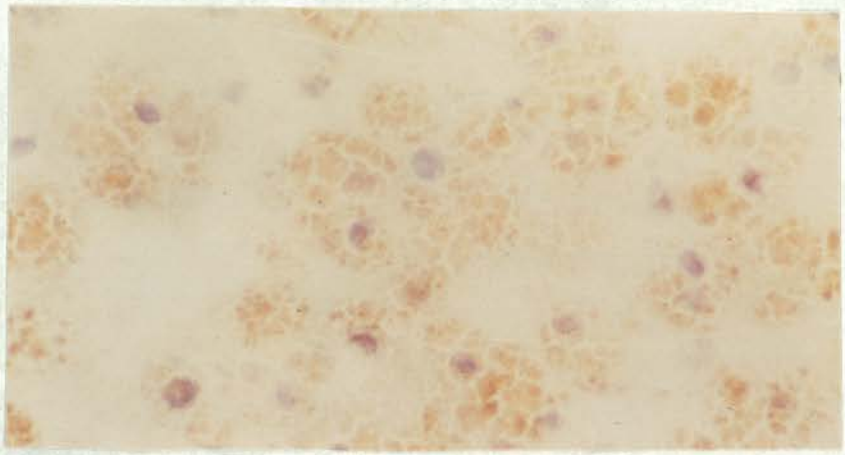


Figure 7 Human adrenal gland stained for DHA sulphate - positive control (frozen section x 800)

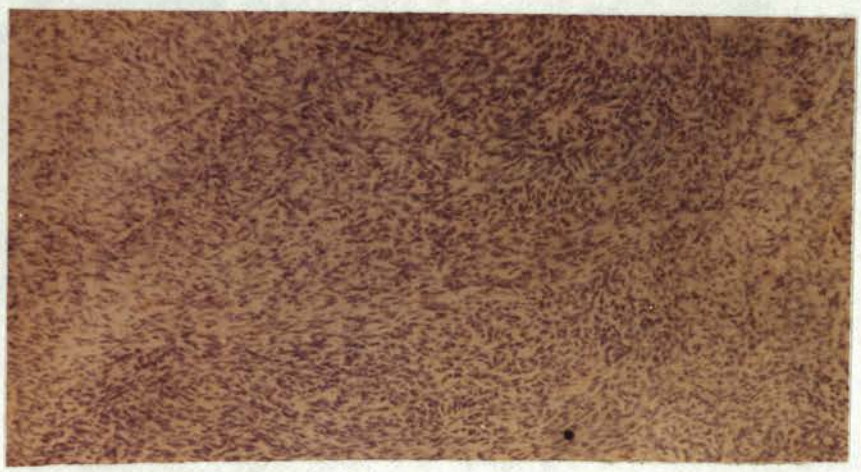


Figure 8 Human ovarian stroma stained for DHA sulphate - negative control (frozen section x 200)

PAS Diastase Stain

Tissues were fixed in 10% formalin solution, processed, embedded in paraffin wax and 3 μm sections cut. These were dewaxed and then incubated for 1 hour at 37°C in 1 g of diastase in 100 ml of distilled water. The slides were then washed in water for 5-10 minutes before being treated with periodic acid (1 g in 200 ml distilled water) for 5 minutes. The tissues were then washed in water and covered with Schiff's reagent for 15 minutes. The slides were then again washed in tap water for 5 to 10 minutes, counterstained with haematoxylin, dehydrated in graded alcohols and xylene and mounted with Permount.

Acid phosphatase

Frozen sections were incubated for 30 minutes at 30°C in a solution of alpha naphthol As B1 phosphoric acid 20 mg, acetone 1 ml, Wolpoles buffer 22.5 ml and hexazotised pararosaniline 0.9 ml. Tissues were then washed in water, counterstained with haematoxylin, dehydrated through graded alcohols and mounted from xylene in permount synthetic resin.

Electron Microscopy

Tissues were fixed in 3% glutaraldehyde in 0.1 m cacodylate buffer overnight. They were then rinsed x 2 in buffer for 30 minutes before being further fixed in 1% osmium tetraoxide. Tissues were then dehydrated through graded alcohols and placed in epoxypropane before being impregnated overnight in araldite. Sections were cut using a glass knife at 900 nm thickness and stained with uranyl acetate before being viewed on a Jeol 1005 electron microscope.

Evidence that Radioimmunoassay is measuring DHA Sulphate

(Miller et al 1980, Miller et al 1982, Miller 1985 personal communication)

(1) If fluids are extracted with ether, then <1% of the material cross-reacting with the antibody is in the aqueous phase. This indicates that the substance being measured is not a free steroid, ie. it cannot be DHA, cortisol, testosterone etc.

(2) Acid solvolysis of fluids followed by ether or ethyl acetate extraction results in the cross-reacting material passing into the organic phase. This indicates the substance being measured is a conjugate.

(3) Saturation of fluids with sodium chloride followed by extraction into an organic solvent drives the cross-reacting material into the organic phase. The substance is therefore not a protein.

(4) On thin layer chromatography the cross-reacting material migrates with DHA Sulphate and following solvolysis migrates with DHA.

(5) The mass spectrum (MS) of the major component of solvolysed extracts after gas liquid chromatography (GLC) closely resembles that of authentic DHA. Quantitative estimates by GLCMS of DHA in solvolysed extracts of plasma and breast secretions are almost identical to those obtained by radioimmunoassay of DHA after a correction has been made for procedural losses. In cyst fluids the values obtained by radioimmunoassay and GLCMS also show reasonable correlation. In some cyst fluids the value of DHA Sulphate was lower, but of the same dimension by GLCMS. In these fluids significant amounts of androsterones sulphate and epiandrosterone sulphate were detected by GLCMS. It is of note that the antibody cross-reacts with these substances (Table 1). Values obtained for cyst fluids therefore principally reflect DHA Sulphate but also include closely related 17 oxo-steroid conjugates, although for simplicity in the text they will be

Introduction

Studies of normal mature non-lactating breasts reveal secretions within lobules, ductules and ducts (Bonser et al 1961). This secretion is seen on staining with haematoxylin and eosin as pale pink material (Figure 1). In periodic acid Schiff stained sections, secretions stain a brilliant violet with paler pink areas (Figure 2). During pregnancy, this staining pattern is lost, indicating that the secretion produced by lactating lobules is different to that produced in the breast of non-lactating breast (Bonser et al 1969). Due to a combination of reabsorption of the secretion and the keratin plugs present in the orifices of the main ducts opening onto the nipple in non-pregnant women, breast secretions in the non-lactating breast rarely escape.

Several investigators have been able to obtain secretions from the nipples of non-lactating women using various types of breast pump (Adair et al 1931, Jackson et al 1951, Papanicolaou et al 1958, Sartorius 1973). These fluids were first used to obtain cells from the breast for cytological analysis in the hope of devising a screening test for breast cancer (Sartorius 1973). However, not all women produce breast secretions. Petrakis et al (1975) investigated the factors which influenced the availability of these secretions. They were able to obtain breast secretions from approximately half of all women. Fluid was obtained from a greater proportion of Caucasian than Chinese women and more commonly from premenopausal women and those under 50 years of age. The phase of the menstrual

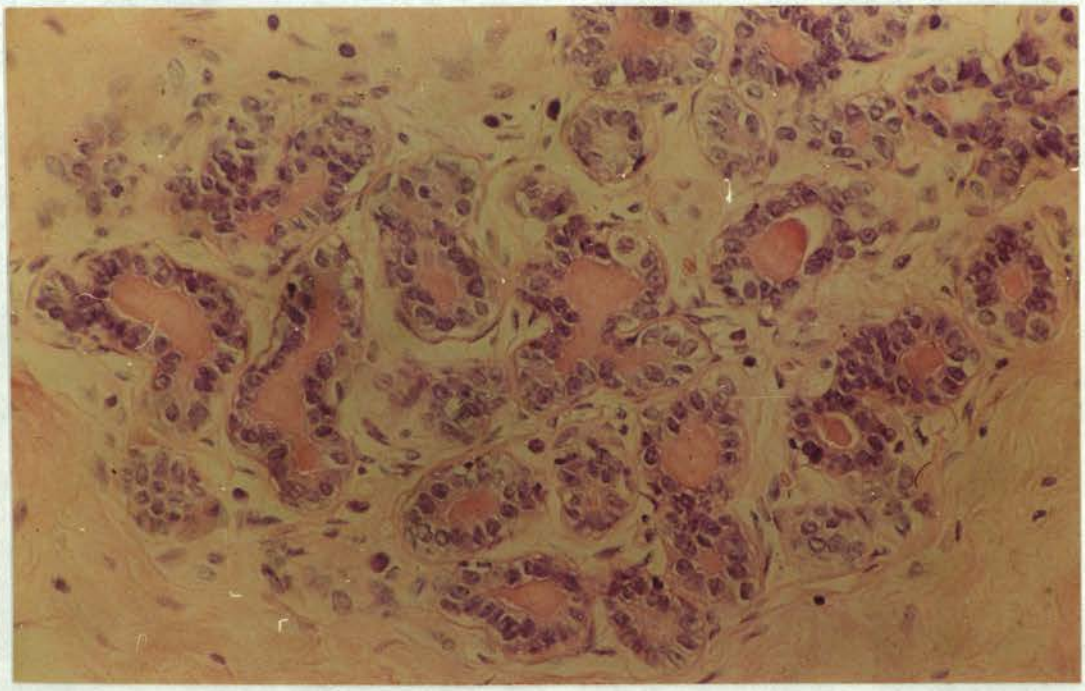


Figure 1 Normal human breast lobule containing lobular secretion (H & E x 200)

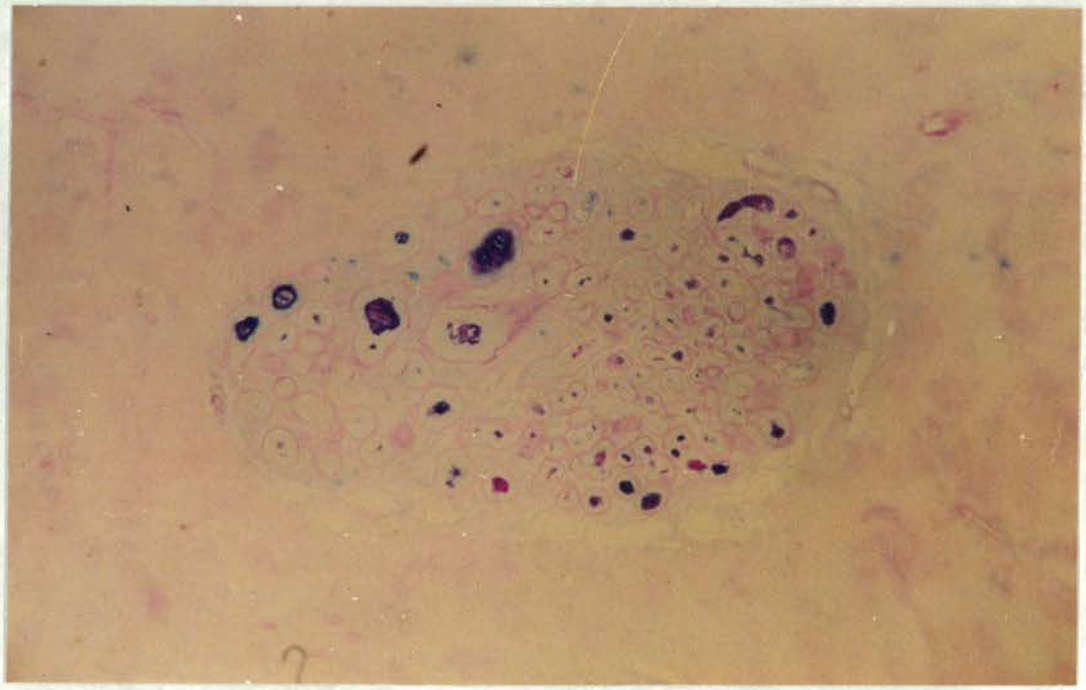


Figure 2 Normal human breast lobule containing lobular secretion. Note the two different colours of secretion indicating two different forms of mucopolysaccharide in the secretion (AB. PAS x 100)

cycle, parity and whether the patient was taking the pill did not have any apparent effect. It was also noted that women with wet type cerumen had a higher incidence of production of breast secretion, indicating that genetic factors may be important in influencing the secretory activity of the non-lactating breast (Petrakis et al 1975).

More recently, the composition of these fluids has been studied. Breast secretions from non-lactating women have been shown to contain high levels of cholesterol, triglycerides, free fatty acids (Petrakis et al 1977c, Petrakis et al 1981), secretory IgA (Petrakis et al 1977b, Yap et al 1981), hormones (Miller et al 1977, Wynder et al 1977, Miller et al 1977, Miller et al 1980, Wynder et al 1981, Miller et al 1983) and, in smokers, nicotine (Petrakis et al 1978), It has been noted that the concentrations of these substances frequently exceed those found in plasma. For example, the androgen conjugate DHA sulphate which, in plasma, is almost exclusively derived from the adrenal cortex, may be present in breast secretions at levels up to 400 times greater than its plasma concentration (Miller et al 1980, Miller et al 1981, Miller et al 1983). It is not known what influences the composition of these secretions, although it has been suggested that hormone composition of these fluids may be related to the pathogenesis of breast disease (Petrakis et al 1975, Wynder et al 1977). It is also of interest that mutagens have been detected in breast secretions with a frequency similar to the incidence of breast cancer in the population (Petrakis et al 1980).

Despite the studies on composition of breast secretions, little is known about the mechanisms involved in the production of these fluids and the factors affecting their composition. It is assumed that they are secreted by the lobular units of the breast and possibly are modified as they pass down the duct system. This is, however, far from proven. The finding of large amounts of DHA sulphate in breast secretions offers a possible marker to study these processes.

Some studies have been performed on DHA sulphate in breast secretions and these have shown that values in any individual are consistent in both breasts and remain constant throughout the menstrual cycle. As yet, no differences in levels of DHA sulphate in breast secretions from normal women, those with benign disease and those with breast cancer, have been detected (Miller et al 1981).

Studies on composition

(i) Measurement of DHA sulphate, Na^+ and K^+ in breast secretions obtained by nipple aspiration

(ii) Immunoperoxidase localisation of DHA sulphate within the normal breast

(iii) Concentration of DHA sulphate in secretions obtained from within the breast parenchyma

(iv) The derivation of DHA sulphate in breast secretions

Studies with Metoclopramide

prednisolone

aminoglutethimide

(i) Measurement of DHA sulphate, Na+ and K+ in breast secretions obtained by nipple aspiration

The aim of this study was to confirm that high levels of DHA sulphate were present in human breast secretions obtained by nipple aspiration and also to measure Na+ and K+ in these fluids.

Patients, Materials and Methods

Sixty breast secretions from 48 patients had DHA sulphate estimated by radioimmunoassay. Na+ and K+ were measured by flame photometry.

Results

The values of DHA sulphate in the 60 breast secretions are shown in Figure 1. All DHA sulphate concentrations in these secretions were above the upper limit of the reference range for plasma. The Na+ and K+ concentrations in these 60 secretions are shown in Figure 2. The range for Na+ was 96-244 mmol/l and for K+ was 5-30 mmol/l. Breast secretions therefore contain higher concentrations of K+ than does plasma; however Na+ concentrations were always greatly in excess of those of K+. This is more clearly demonstrated when the ratio of Na+ to K+ in breast secretions is plotted (Figure 3).

There was no significant correlation between the values of DHA sulphate, Na+ and K+.

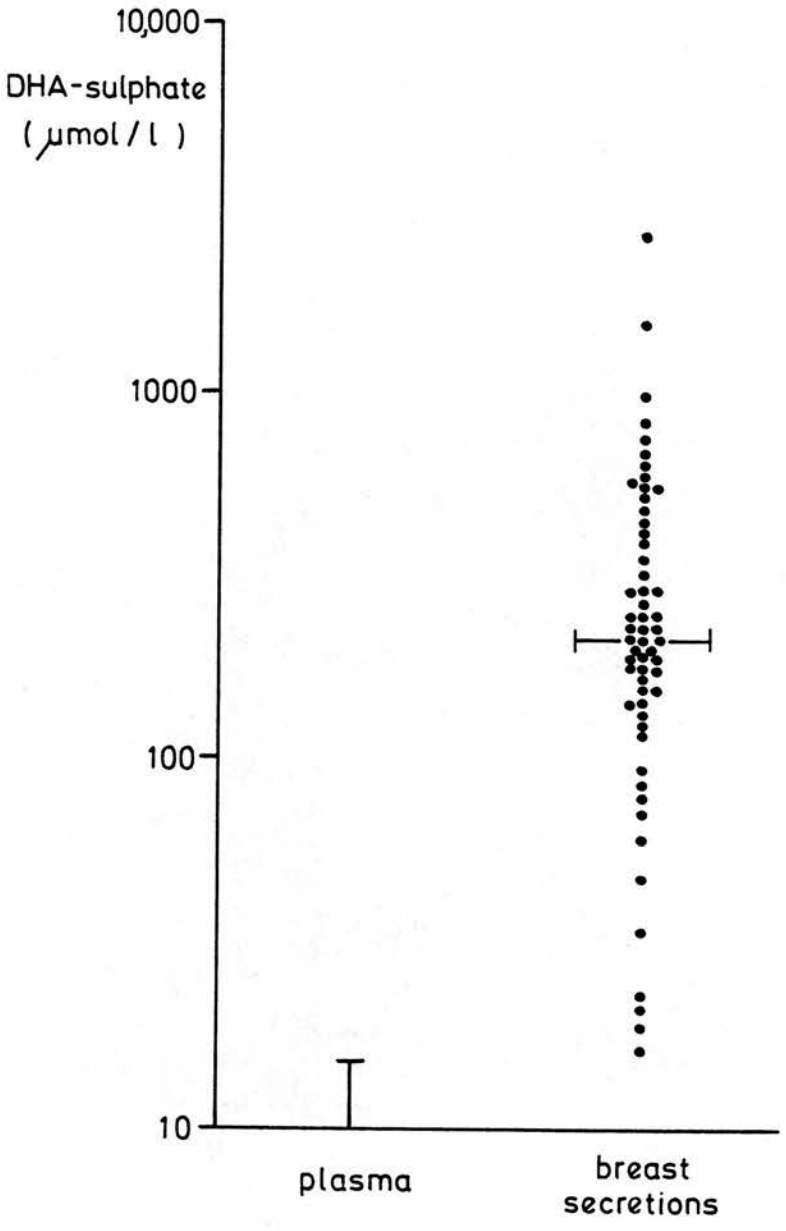


Figure 1 Concentration of DHA sulphate in human breast secretions obtained by nipple aspiration (horizontal bar represents median value)

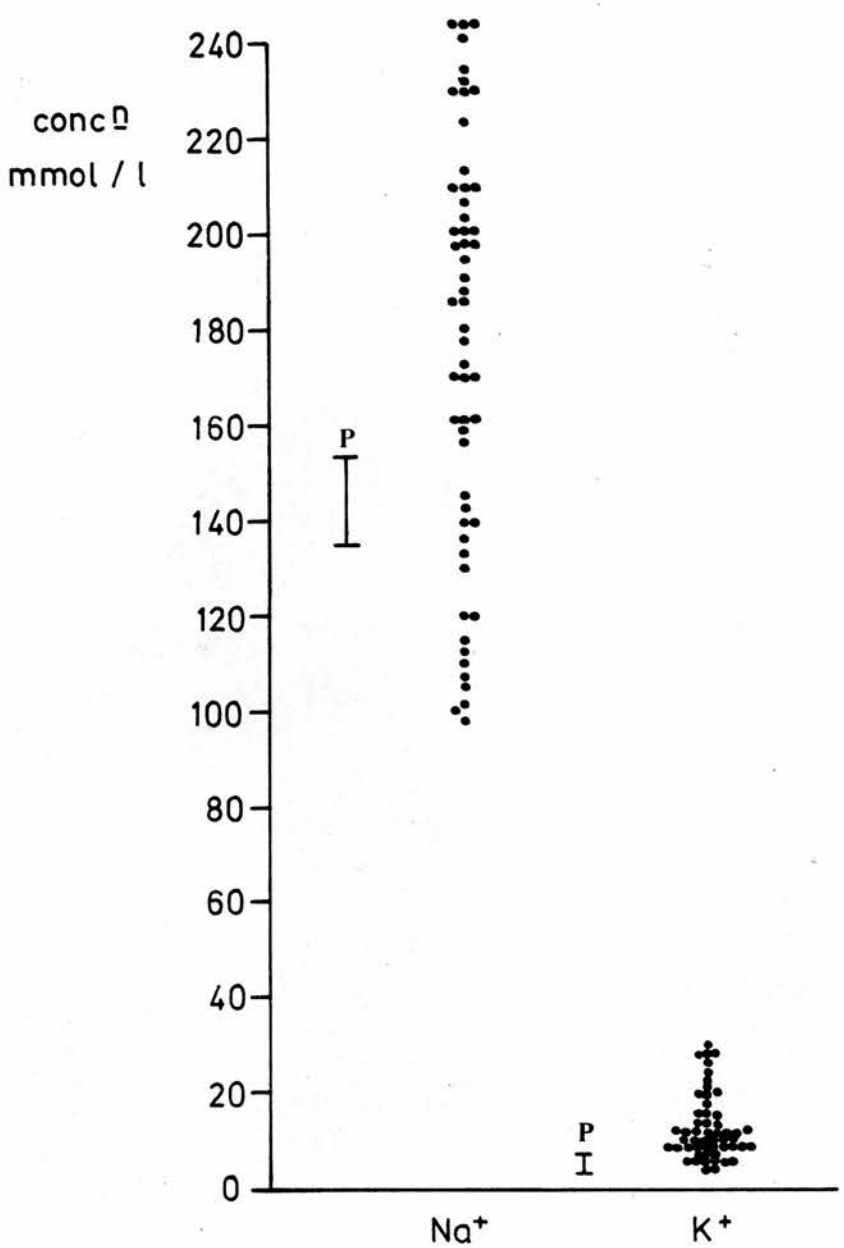


Figure 2 Concentration of Na+ and K+ in breast secretions obtained by nipple aspiration
P= Plasma range

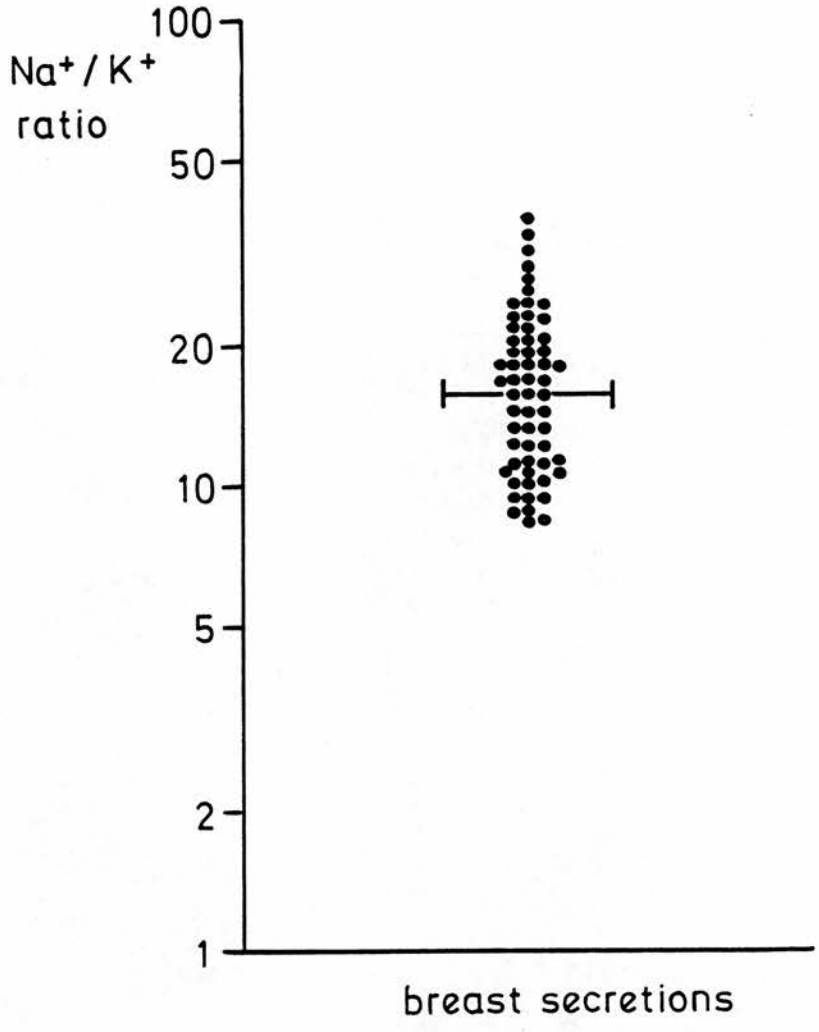


Figure 3 Na+/K+ ratio in breast secretions obtained by nipple aspiration

(ii) Concentration of DHA sulphate in secretions from within the breast parenchyma

It is considered that breast secretions obtained from the nipple are derived from secretion by the lobular units of the breast and are modified as they pass through the duct system. The aim of the present study was to determine the concentration of DHA sulphate from fluid obtained from terminal and interlobular ducts and from major ducts leading to the nipple.

Materials and Methods

Seven mastectomy specimens from seven patients with breast carcinoma were carefully sectioned into thin slices and examined under a dissecting microscope. Secretions were obtained from visible terminal and interlobular ducts (Figure 4) and collected into calibrated capillary tubes at sites distant from the breast cancer. Secretions from larger ducts were visible macroscopically and collected in a similar manner.

The secretions were diluted in distilled water and stored at -40°C for later analysis. DHA sulphate was measured in each sample by radioimmunoassay. Comparison of DHA sulphate concentrations in the two groups was by the Wilcoxon Rank Sum test.

Figure 4 The interlobular ducts of the breast as seen under the dissecting microscope (x 25)

Results

Fourteen samples of secretions from terminal breast ducts were obtained from the seven specimens. The volumes obtained ranged from 0.5 - 3.5 µl . Nine secretions were obtained from larger ducts, the volumes ranging from 1 - 13 µl .

The concentrations of DHA sulphate in these two groups of secretions are shown in Figure 5. All values for DHA sulphate in the major duct secretions were higher than those secretions obtained from terminal ducts. The difference between the groups was also statistically significant $p < 0.01$. The concentration of DHA sulphate within the major ducts is similar to that reported to breast secretions obtained from the nipple, shown in Figure 1.

The ratio of DHA sulphate in secretions from terminal ducts to that in the major ducts was not constant, but varied from approximately 3 to 15 times. Thus, the concentrations of DHA sulphate within the major ducts do not reflect the concentration deep within the breast and it is not possible to determine this concentration by knowing the value in the major ducts.

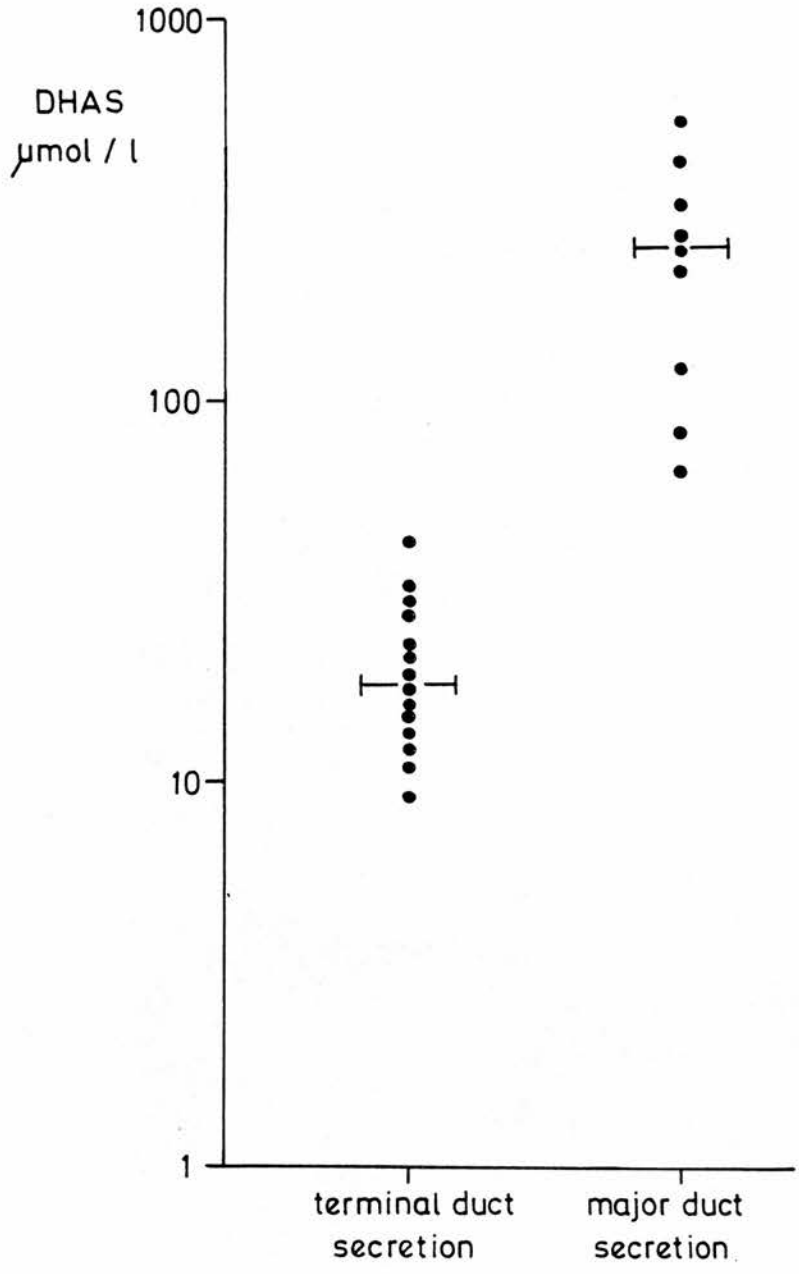


Figure 5 Concentration of DHA sulphate in small and large ducts within the breast

(iii) Immunoperoxidase localisation of DHA sulphate in the normal breast

The aim of this study was to localise DHA sulphate within lobules, ducts and connective tissues within the normal breast.

Materials and Methods

Frozen sections of normal breast were obtained from bilateral reduction mammoplasties performed in two patients. DHA sulphate was localised in frozen sections by the method previously described.

Results

The secretions in normal breast lobules contained material cross-reacting with the antibody (Figure 6), thus suggesting that these contain concentrations of DHA sulphate greater than in plasma or surrounding tissues. There was little or no apparent staining within the cytoplasm of normal breast lobular epithelium. There was no localisation of DHA sulphate to intralobular or interlobular stroma. The secretions in breast ducts also contained large amounts of materials cross-reacting with the antibody and the staining appeared deeper than in the lobules, possibly indicating a higher concentration of DHA sulphate within ductal secretions (Figures 7 and 8). Staining was also seen related to ductal epithelium (Figure 9). It was uncertain whether this was present in the cytoplasm of these cells or between the cells.

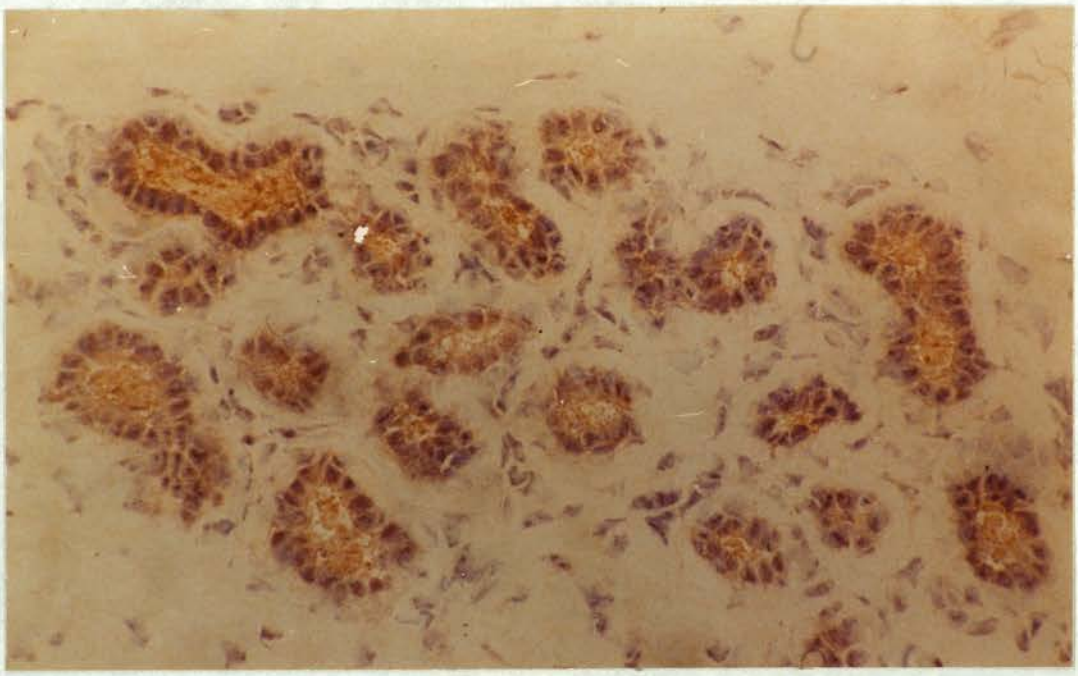


Figure 6 Normal breast lobule stained by the immunoperoxidase technique to demonstrate DHA sulphate. Note the staining in the lobular secretions.

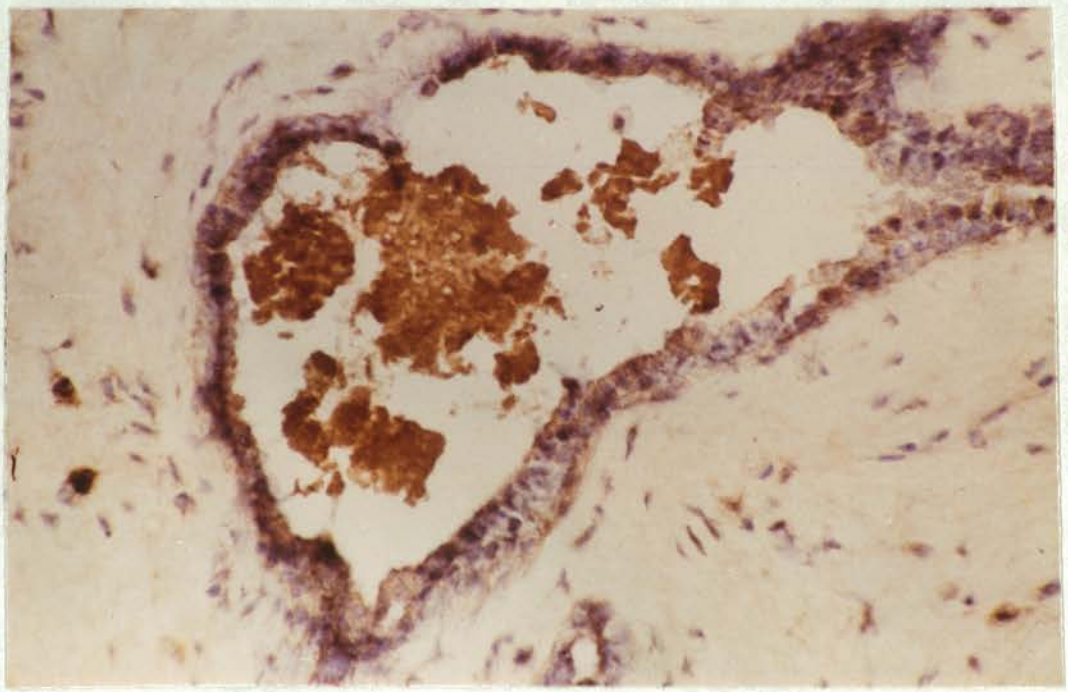


Figure 7 Normal breast stained to demonstrate DHA sulphate. Note the deeper staining within breast ducts.

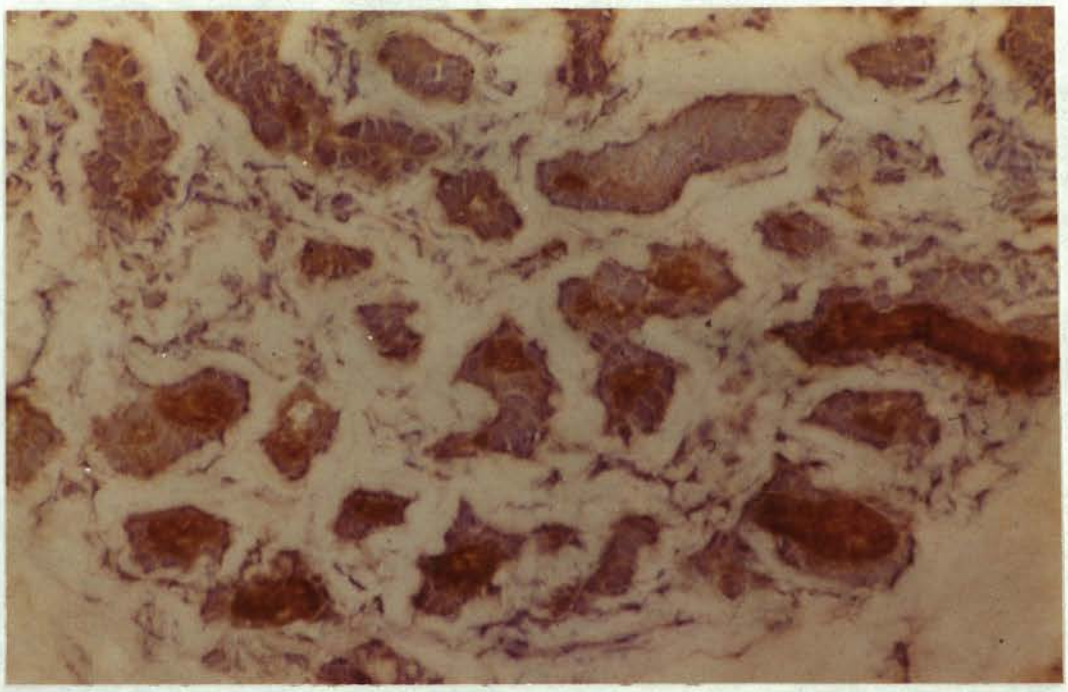


Figure 8

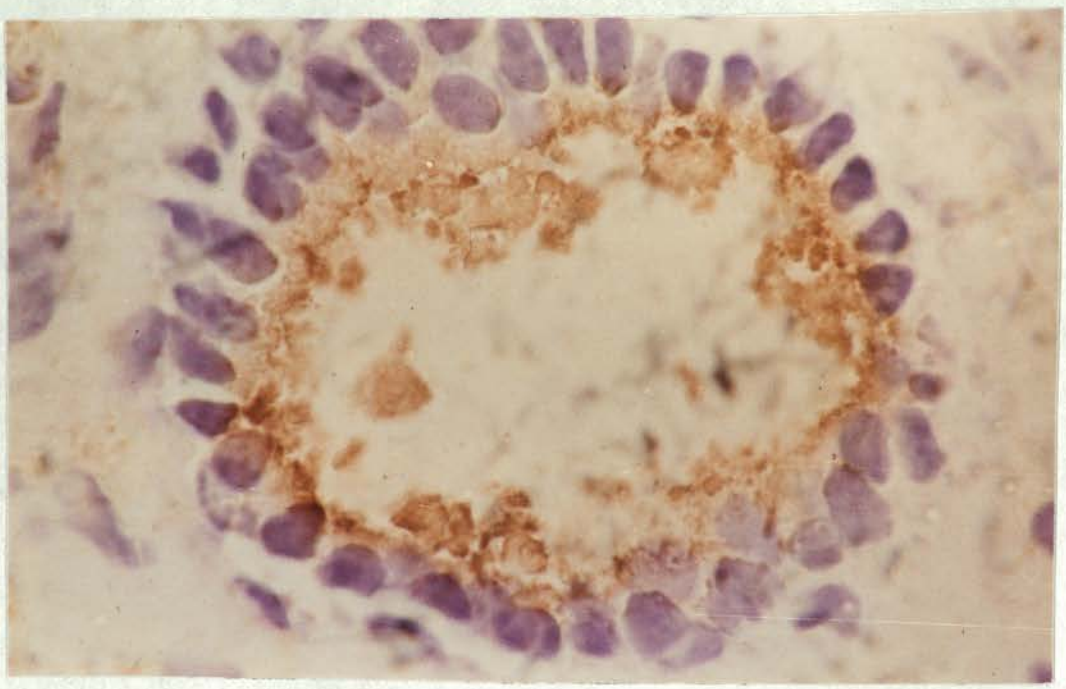


Figure 9

Further examples of DHA sulphate within the breast visualised by the immunoperoxidase technique - note the staining between the ductal epithelium in Figure 9

(iv) The derivation of DHA sulphate in breast secretions

Studies with Metoclopramide

prednisolone

aminoglutethimide

Introduction

The aim of the present study was to try and determine whether the DHA sulphate present in breast secretions is concentrated from plasma or synthesised within the breast. To attempt to answer this question, breast secretions obtained by nipple aspiration and plasma from normal women and patients with breast cancer have been collected on different occasions after taking pharmacological agents which have been reported to increase or decrease plasma levels of DHA sulphate.

Women with hyperprolactinaemia may have elevated levels of DHA sulphate (Bassi et al 1977, Guist et al 1977, Vermeulen et al 1977, Vermeulen et al 1978). Drug induced hyperprolactinaemia has similarly been reported to be associated with increased plasma DHA sulphate concentrations. Metoclopramide in some, but not all patients, produces hyperprolactinaemia (Schyve et al 1978, Vermeulen et al 1978). The administration of metoclopramide may therefore be expected to produce an increase in plasma levels of DHA sulphate.

The administration of corticosteroids in pharmacological doses suppresses adrenal output and thus reduces the circulating level of DHA sulphate. Aminoglutethimide, an agent used in the treatment of breast cancer, given in combination with cortisol replacement, reduces levels of plasma oestrogens and precursors (Santen et al 1980, Mason 1981). Plasma DHA sulphate levels are reduced to less than 5% of their pre-treatment value.

Patients, Materials and Methods

Two groups of four normal women were studied before, during and after treatment with either prednisolone 15 mg/day or metoclopramide 30 mg/day according to the following protocol:

Day 0 : Plasma and breast secretions collected.
Day 1 : Either prednisolone or metoclopramide started.
Day 8 : Plasma and breast secretions collected.
Drug stopped.
Day 15 : Plasma and breast secretions collected.

Two patients with breast cancer taking aminoglutethimide 1 g/day and hydrocortisone 40 mg/day had plasma and secretions collected before and between 14 and 21 days after treatment.

DHA sulphate concentrations were measured in all samples of plasma and breast secretions by radioimmunoassay.

Results

Metoclopramide had no significant effect on the concentration of DHA sulphate either in plasma or in breast secretions (Figure 10).

Prednisolone markedly reduced plasma DHA sulphate levels and also lowered the concentration of DHA sulphate in breast secretions, although this effect was not as great as that in plasma (Figure 10). It was also evident that the post-prednisolone samples of plasma and secretions did not attain the same concentration of DHA sulphate as in the original specimens.

Aminoglutethimide produced more marked falls in the level of DHA sulphate in both plasma and secretions (Figure 11). As with prednisolone, the effect was greater in plasma.

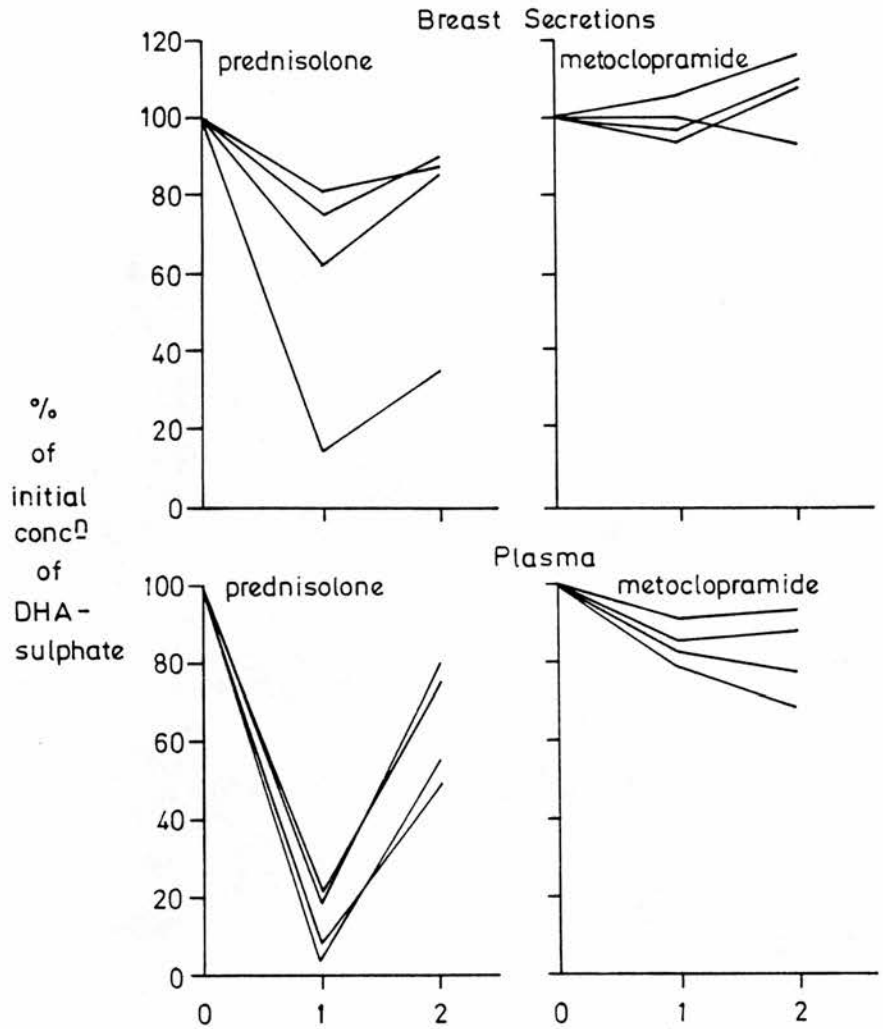


Figure 10 Effect of Metoclopramide (Maxolon) and Prednisolone on the concentration of DHA sulphate in plasma and breast secretions (0 - time zero, 1 - drug for 1 week, 2 - 1 week after stopping drug)

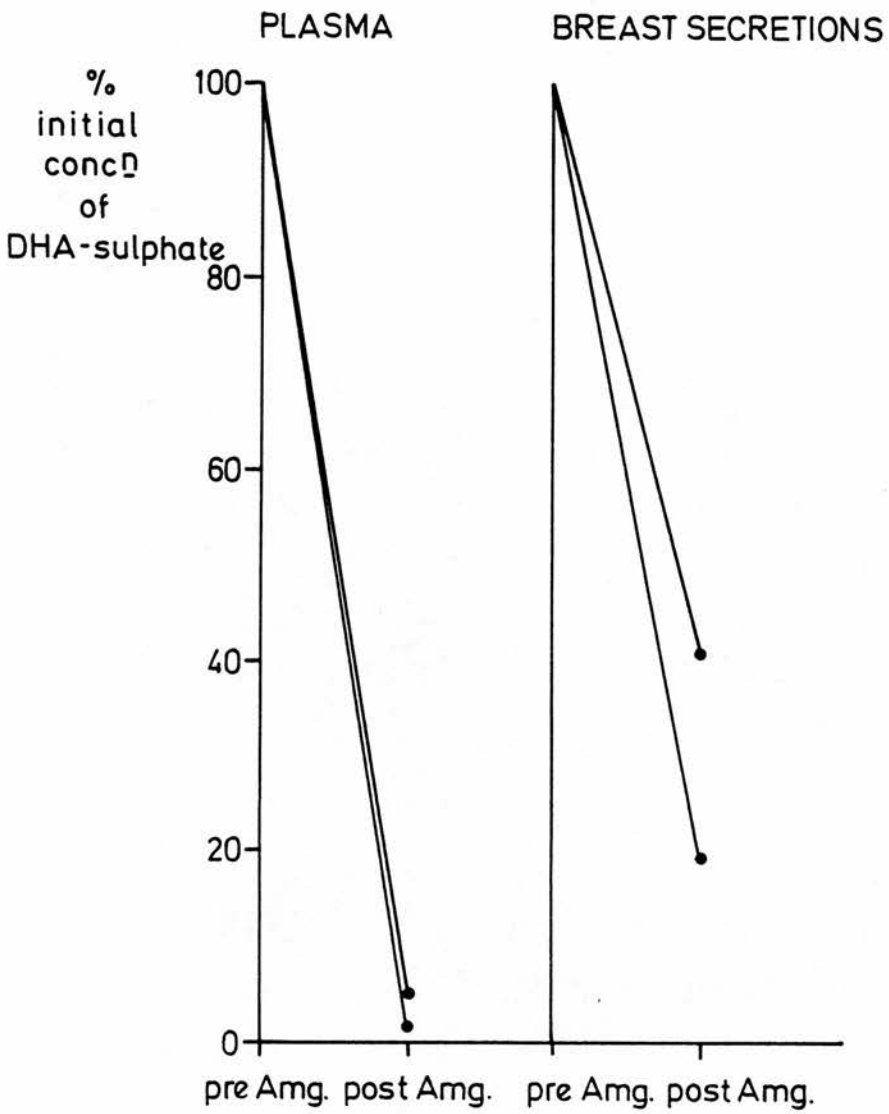


Figure 11 Effect of Aminoglutethimide on the concentration of DHA sulphate in breast secretions

DISCUSSION

The secretions produced by the human breast lobular epithelium have been shown in the present study to contain concentrations of DHA sulphate many times greater than those in plasma. This concentration increases as the secretion passes up to the nipple, presumably as a result of reabsorption of fluid. The degree of this increase appears to vary in different breasts and thus measurement of DHA sulphate in secretions obtained from the nipple will not directly reflect the concentrations present within the breast lobules, although it may give a more accurate assessment than measurement of DHA sulphate in plasma. It is in the lobules that the majority of breast diseases arise and it may be that the only concentrations of hormones which are important in relation to the aetiology of breast disease are those in contact with these cells. This may explain why studies of hormones in plasma, urine and in secretions obtained by nipple aspiration have failed to show consistent differences between groups of normal women and those with breast disease.

The sources of DHA sulphate in breast and apocrine secretion is unknown. The present study has shown that reduction of plasma DHA sulphate levels by the administration of drugs results in a decrease in DHA sulphate concentrations within breast secretions. The fall was less marked than in plasma but it may be that a more prolonged course of treatment would have resulted in a further lowering of levels in secretions. This is supported by the greater reduction in

DHA sulphate in the patients taking aminoglutethimide who were studied between 2 and 3 weeks post-treatment. These findings suggest that DHA sulphate may simply be concentrated from plasma sources in a similar manner to the entry of DHA sulphate into breast cyst fluid (Bradlow et al 1981a, Bradlow et al 1983a). However, both prednisolone and aminoglutethimide reduce the concentrations of other steroid hormones and the present observations do not rule out the conversion of other steroid hormones to DHA sulphate within the lobular epithelium.

The breast is said to be a modified apocrine sweat gland (Petrakis et al 1977a). Secretion from apocrine sweat glands has also been noted to contain high concentrations of DHA-sulphate (Labows et al 1979). The concentration of DHA-sulphate within the secretion in the breast lobules may therefore represent the degree of apocrine secretory activity of the breast. It is of note that the degree of apocrine activity in the breast has been related to the risk of developing breast cancer (Petrakis et al 1977a).

The role of DHA and its sulphate within secretions remains unknown. It is known that these compounds can be converted to both active androgens and active oestrogens. This has been demonstrated in normal, benign and malignant breast tissue (Abul-Hajj 1975, Miller et al 1979, Miller 1984). It may be that a variation in concentrations of these substrates may relate to the local levels of active hormones. Thus the levels of DHA sulphate in breast lobular secretion may be of importance in the aetiology of breast disease.

BREAST CYSTS AND CYSTIC DISEASE

Introduction

Cystic disease of the breast is a term used to cover the condition in which palpable cysts are the dominant feature. These may be accompanied by several microscopic lesions including (i) microcysts; (ii) apocrine epithelium; (iii) adenosis; (iv) hyperplasia; and (v) fibrosis. Some 7% of all women in the Western world develop a palpable breast cyst (Haagensen et al 1981). The true incidence of cystic disease, as determined in post-mortem studies, is approximately 20% for macroscopic cysts and 55% for microcysts (Frantz et al 1951, Davis et al 1964). It is thus by far the most common benign disease of the breast. Despite its frequency, little is known of the origin, mode of formation and content of breast cysts. This is perhaps even more surprising in view of the association between cysts and epithelial hyperplasia and the suggestion that patients with cystic disease may be an increased risk of subsequent breast cancer.

A more detailed consideration is now presented of the origin, mode of formation and composition of human breast cysts and the relationship of cystic disease to hyperplasia and breast cancer.

Origin and mode of formation

Astley Cooper (1829) first distinguished cysts from malignant transformations. The first comprehensive clinical and pathological descriptions of cystic disease were made over half a century later

by Reclus (1883) and Brissaud (1884). Reclus showed that his "maladie kystique des mammelles" was frequently bilateral, might involve the whole of the gland and that most cysts were impalpable. He regarded cysts as independent new formations. In contrast, Brissaud (1884) thought cysts arose by proliferation of lobular epithelium to form compact cellular masses, which became cystic following central necrosis. This theory was later supported by Schimmelbusch (1890). König (1893) considered cysts to be inflammatory in origin and called the condition chronic cystic mastitis, a term which remains in common use.

During the early part of the twentieth century, it was believed that cysts arose as a consequence of senile involution in the breast (Tietze 1900, Bloodgood 1906, McFarland 1922). An extreme proponent of the involutional theory was McFarland (1922), who found cysts only in breasts of women who had lactated and considered them as variations of the involutional process of lactational lobules. Other workers could find no evidence of residual lactational lobules (Semb 1928) and returned to the theory originally proposed by Reclus (1883) that cysts were new formations arising by hyperplasia.

The role of apocrine epithelium in cystic disease was first recognised by Creighton (1902). The observation that cysts were frequently lined by this epithelium led to the view that cysts were derived from apocrine sweat glands. These glands were thought by some to be normally present in the breast (Nicholson 1923, Ewing 1940) and by others to be present as a result of a developmental

anomaly (Krompecher 1913, Von Saar 1907). Studies have now shown that apocrine epithelium is not present within the breast in early life and may be found in direct continuity with normal ductal epithelium, making the theory of a sweat gland origin for cysts untenable (Cheatle et al 1931, Dawson 1932, Bonser et al 1961). It is now accepted that apocrine epithelium in the breast arises as a result of a transformation from normal duct or lobular epithelium (Bonser et al 1961). This transformation may or may not be a true metaplasia (Dawson 1932, Lendrum 1945, Haagensen et al 1981). It is also now appreciated that, although the majority of cysts are lined by apocrine epithelium, some are lined by flattened epithelium (Azzopardi 1979, Haagensen et al 1981). It has been suggested that simple flattened cysts arise by lobular dilatation, whereas apocrine cysts arise from lobules in which apocrine change is already present (Wellings, Jensen and Marcum 1975, Wellings 1980). This is disputed by others who believe that all cysts in their initial stages (microcysts) are apocrine (Haagensen 1983).

Thus, whilst it is now considered that cysts arise from lobules, their mode of formation and the nature of their precursors remains uncertain.

Relationship to hyperplasia and breast cancer

The association of epithelial proliferation and cysts is so well recognised that it is accepted as a component feature of cystic disease (Azzopardi 1979, Haagensen et al 1981). Proliferation may

take two forms: (i) increase in the number of lobular units (adenosis); or (ii) increase in the number of cells lining the existing lobules (hyperplasia) (Azzopardi 1979). It is hyperplasia that has been shown to be associated with an increased risk of breast cancer (Black et al 1972, Kodlin et al 1977, Page et al 1978), although the significance of this risk remains controversial.

Astley Cooper (1829), when he first described cysts, noted that carcinoma could coexist in the same breast, a finding confirmed by Brodie (1946). Early workers considered these carcinomas to be incidental findings (König 1893). Tietze (1900) was the first to regard the "adenomatous" proliferation seen in cystic disease to be precancerous. Since then, numerous studies have attempted to analyse the interrelationship of cystic disease, hyperplasia and breast carcinoma.

The types of evidence relating cystic disease and carcinoma include:

(i) a similar relationship to parity Both are more common in nulliparous women, possibly suggesting a common aetiological factor (Bonser et al 1961).

(ii) demonstration of the initial stages of carcinoma in cysts or associated hyperplasia Carcinoma can arise in the wall of a cyst, although it is rare (Cheatle et al 1931, Bonser et al 1961). Carcinoma may arise in apocrine epithelium (Borst 1904, Krompecher 1913, Berka 1911, Cheatle et al 1931, Dawson 1932, Lee et al 1933,

Ewing 1940, Geschickter 1943, Stewart 1950, Azzopardi 1979) and about 10% of all invasive carcinomas demonstrate marked apocrine differentiation (Bonser et al 1961, Dixon et al 1983).

(iii) incidence of cystic disease in patients with carcinoma Where adequate data on the incidence of cystic disease in the general population have been considered, results from these studies have been conflicting (Foote et al 1945, Frantz et al 1951, Bonser et al 1961, Sandison 1962, Davis et al 1964). It is accepted, however, that there is a greater incidence of hyperplasia in breasts affected by carcinoma, suggesting, but not proving, that such lesions may be the precursors of malignancy.

(iv) retrospective studies Foote and Stewart (1945), in a retrospective case control study, showed patients with breast cancer had a 2.2 times greater incidence of a previous benign breast biopsy.

(v) prospective studies The majority of these studies have reviewed benign biopsies performed many years ago and the subsequent course of patients observed. The majority suggest some increased risk of breast cancer in patients having a biopsy for cystic disease, the overall risk being between 1.5 and 4.5 times the expected (Warren 1940, Davis et al 1964, Donnelly et al 1975, Monson et al 1976, Page et al 1978). These studies have indicated that it is epithelial hyperplasia that is associated with the increased risk (Black et al 1972, Kodlin et al 1977, Page et al 1978). Haagensen (1971), in

contrast, suggests gross cysts rather than epithelial proliferation are the pathological feature associated with this increased risk. In keeping with this, he has shown that patients with cysts treated by aspiration have an increased subsequent risk of breast cancer compared to the general population, the risk being greater in patients who have two or more cysts (Haagensen et al 1981).

(vi) specific histological features in the breasts of patients with cancer Wellings et al (1975) found a higher incidence of many lesions in the breasts of patients with breast cancer. Both Wellings (1975) and Izuo (1971) showed apocrine change and apocrine proliferation to occur more frequently in association with breast cancer.

In conclusion, there remains conflicting views expressed in the literature regarding the risk of carcinoma in patients with cystic disease. The generally held view is that there is a definite, statistically valid, but modest increased risk in such patients (Azzopardi 1979).

Composition of Cyst Fluids

A variety of electrolytes, proteins and hormones have been measured in breast cyst fluids.

Electrolyte concentrations have been shown to vary widely between individual cyst fluids (Gatsy et al 1979, Bradlow et al 1981b, Bradlow et al 1983b). This variation greatly exceeds that in plasma and the concentrations of sodium and potassium range from those of extracellular fluid ([Na+] >> [K+]) to those of intracellular fluid ([K+] > [Na+]). Attempts have been made to classify cyst fluids on the basis of these cations (Bradlow et al 1981b, Bradlow et al 1983b, Gairard et al 1983).

Numerous proteins in cyst fluid have been studied. The total protein content of these fluids tends to be less than that of plasma (Gairard et al 1983), but specific proteins occur in concentrations greatly in excess of those in plasma. These proteins include carcinoembryonic antigen (Fleisher et al 1974, Gairard et al 1983), a progesterone binding protein (Pearlman et al 1973) and a series of glycoproteins (Haagensen et al 1977, Haagensen et al 1979). Immunoglobulins have also been measured and characterised in cyst fluid. IgA may exist in the 7S or 11S form, the predominant form varying between individual fluids (Yap et al 1982, Yap et al 1984). Fluids with the secretory form of IgA (11S) have been shown to have

higher concentrations of DHA sulphate and lower concentrations of albumin than those with the 7S form (Yap et al 1982, Yap et al 1984).

The hormonal composition of cyst fluid has been extensively studied. Of the peptide hormones GH, LH and FSH are usually found in lower concentrations in cyst fluid than in plasma, whereas calcitonin, prolactin and human chorionic gonadotrophin are present in greater amounts in cyst fluids (Bradlow et al 1983a, Melis et al 1983). Steroid hormones can be divided into two groups. Unconjugated hormones such as corticosterone, progesterone, testosterone, oestrone and oestradiol do not accumulate significantly in cyst fluids, the median value in cyst fluids being approximately three times that of plasma. On the other hand, androsterone, epiandrosterone and dehydroepiandrosterone and their conjugates are present in concentrations many times than those in plasma (Bradlow et al 1976, Bradlow et al 1979, Bradlow et al 1981a, Miller et al 1982, Bocuzzi et al 1983, Bradlow et al 1983a, Melis et al 1983, Miller et al 1983). The hormone conjugate DHA sulphate may be present in cyst fluids in amounts many hundred times its plasma level (Miller et al 1982, Bradlow et al 1983a). It is of note that DHA sulphate is not similarly concentrated in cyst fluids of renal, ovarian or hepatic origin (Bradlow et al 1983a).

Tracer studies using labelled steroids have shown that, of those steroids present in cyst fluids in high concentration, only DHA sulphate shows any significant accumulation in cyst fluid. These

studies also demonstrated that neither $^{3}H_2O$ or ^{14}C antipyrene readily enters cyst fluid, indicating that the entry of DHA sulphate into cyst fluids must be facilitated by some mechanism such as active transport (Bradlow et al 1983a).

Despite the variety of substances that have been measured, our understanding of how cyst fluids form and what influences their composition, or if the composition relates to subsequent behaviour of cystic disease, remains ill understood.

Studies on the Composition of Human Breast Cyst Fluid

(i) Electrolytes and DHA sulphate

(ii) Proteins and form of IgA

(iii) pH

(iv) Factors affecting composition of cyst fluid

a) age and menopausal status

b) volume

c) multiplicity

d) oral contraceptives

e) length of history

(v) Correlation of concentration of DHA sulphate in breast cyst fluid and breast secretions in the same patient

(vi) Correlation of composition of cyst fluid and frequency and volume of breast secretions

(i) Electrolyte and DHA sulphate concentrations in breast cyst fluid

The aim of this study was to measure Na+, K+ and DHA sulphate in breast fluids and to analyse their interrelationships to assess whether it might be possible to classify cyst fluids on the basis of these constituents.

Patients, Materials and Methods

One hundred cyst fluids were obtained by needle aspiration from 85 patients. In 75 subjects a single cyst was aspirated and in the 10, multiple cysts were drained. Volumes aspirated varied from 0.7 to 50 ml. All 100 patients were premenopausal.

Na+ and K+ were measured by flame photometry and DHA sulphate by radioimmunoassay.

Analysis of data was performed by the Kendall Rank or Wilcoxon Rank sum test.

Results

The levels of Na+ and K+ in the 100 cyst fluids are shown in Figure 1. Values for both ions varied enormously and for K+, were usually greatly in excess of the reference range for plasma. The distribution of values about the mean for both Na+ (Figure 2) and K+

(Figure 3) was suggestive of there being more than one population. This was accentuated when the ratios of Na+ to K+ in the 100 cyst fluids were examined (Figure 4).

The concentrations of DHA sulphate in each cyst fluid is shown in Figure 5. Levels varied from 1.5 to 870 μmol/l with a median value of 80 μmol/l.

The interrelationships between Na+, K+ and DHA sulphate are shown in Figures 6, 7 and 8. The relationships of Na+ to K+ and Na+ to DHA sulphate were inverse, while K+ and DHA sulphate were directly related. All interrelationships were significant, $p < 0.001$.

As the distribution of the ratios of Na+ to K+ suggested that there were two or three separate populations of cyst fluids, the 100 fluids were arbitrarily separated into three groups on the basis of the Na+/K+ ratio (Figure 9). One group was of 47 fluids in which the Na+ concentration exceeded that of K+, (K+ fluids), another of 43 fluids in which the Na+ concentration was at least three-fold higher than that of K+ (Na+ fluids) and a further group of 10 fluids with intermediate electrolyte values(mix cyst fluids). Values of DHA sulphate in these subgroups were then compared (Figure 10). Concentrations of DHA sulphate in K+ cyst fluids were significantly higher than that in the Na+ group ($p < 0.0005$). Levels in mix cysts were similar to those in the K+ group but significantly higher than the values in the Na+ cysts ($p < 0.001$).

As the mix group was indistinguishable from the K+ group in terms of DHA sulphate concentration, in all further analyses cyst fluids were divided into just 2 groups - those with a low Na+/K+ ratio (<3) and those with a high Na+/K+ ratio (≥3) (Figure 11). Replotting of the data for DHA sulphate (Figure 12) showed little overlap in values in these two groups.

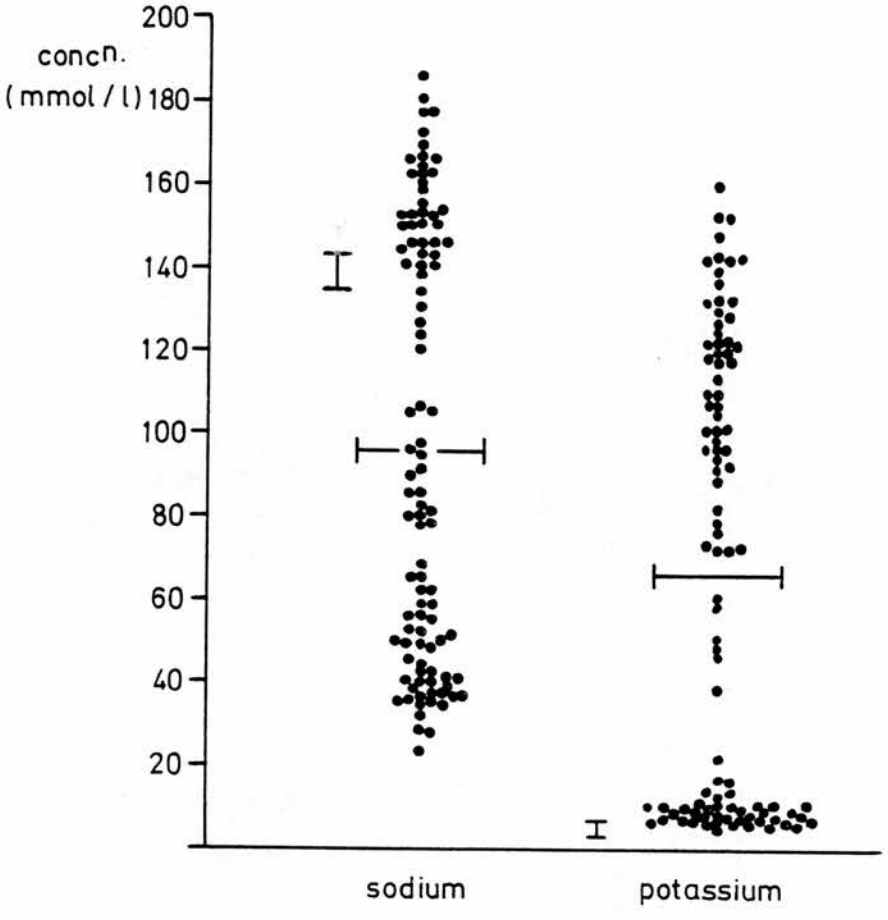


Figure 1 Concentrations of sodium and potassium in human breast cyst fluids. Horizontal lines represent mean level. Vertical lines represent reference range for plasma.

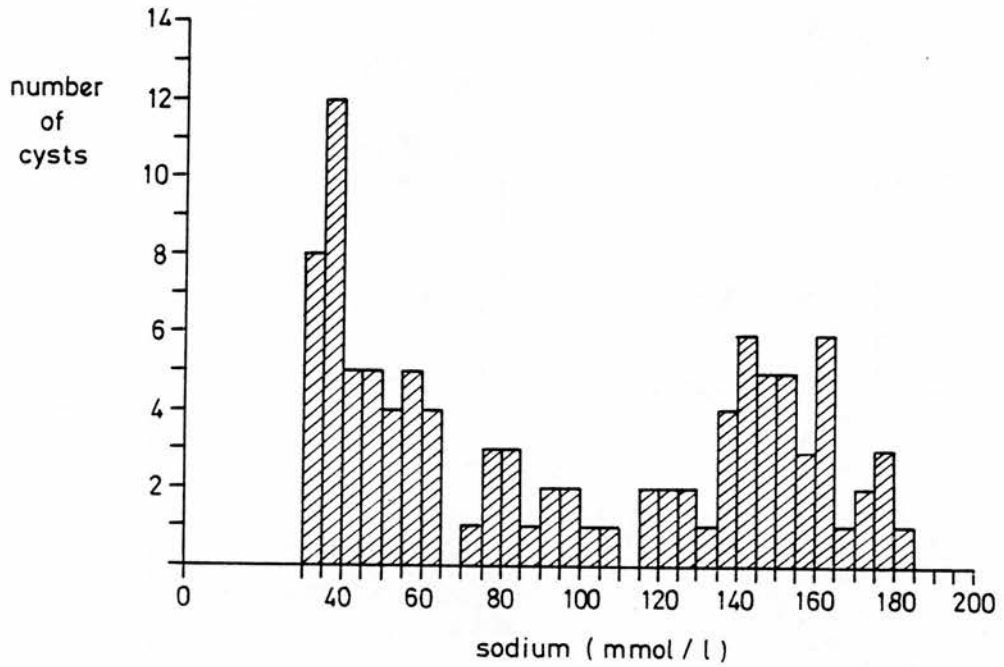


Figure 2 Distribution of the concentrations of sodium in human breast cyst fluids.

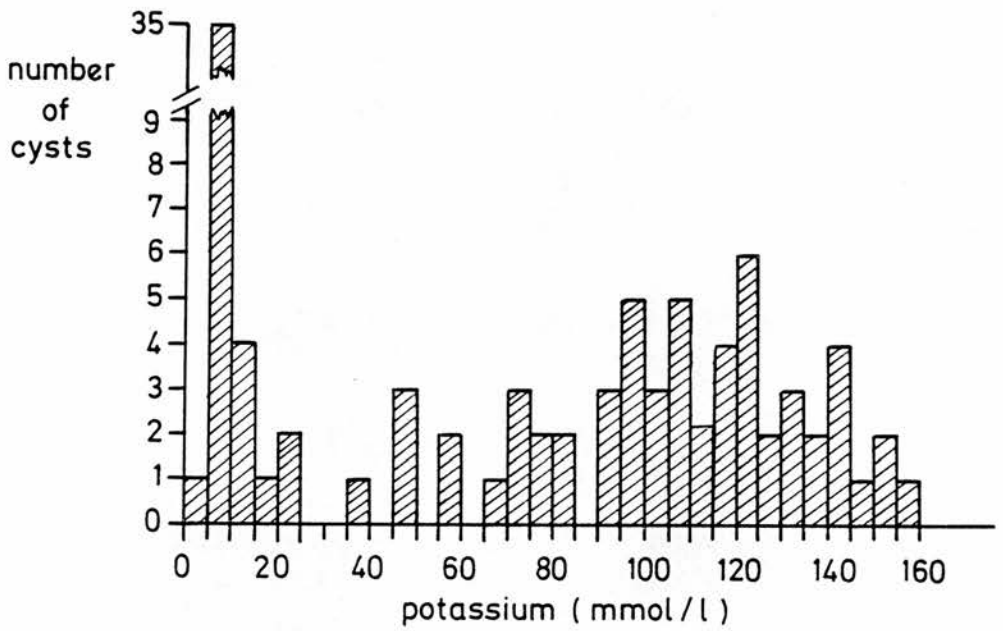


Figure 3 Distribution of the concentrations of potassium in human breast cyst fluids.

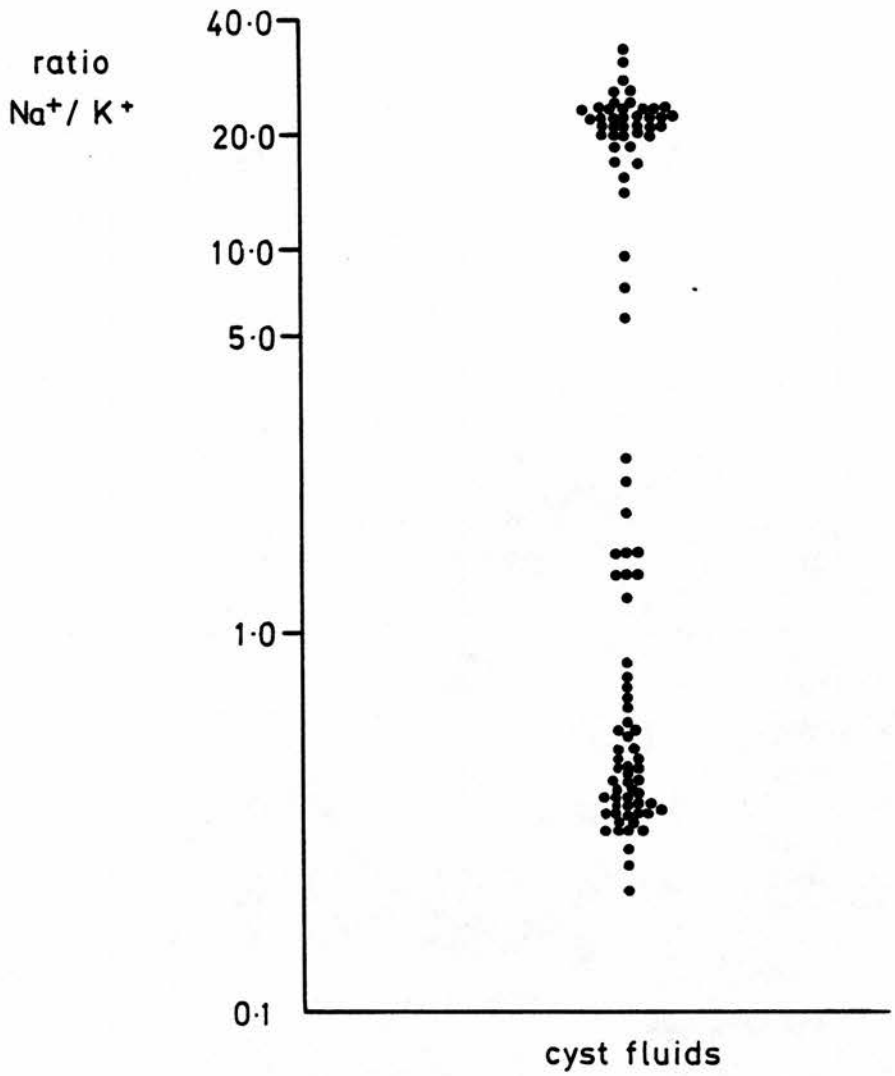


Figure 4 Ratio of sodium to potassium (Na+/K+ ratio) in breast cyst fluids.

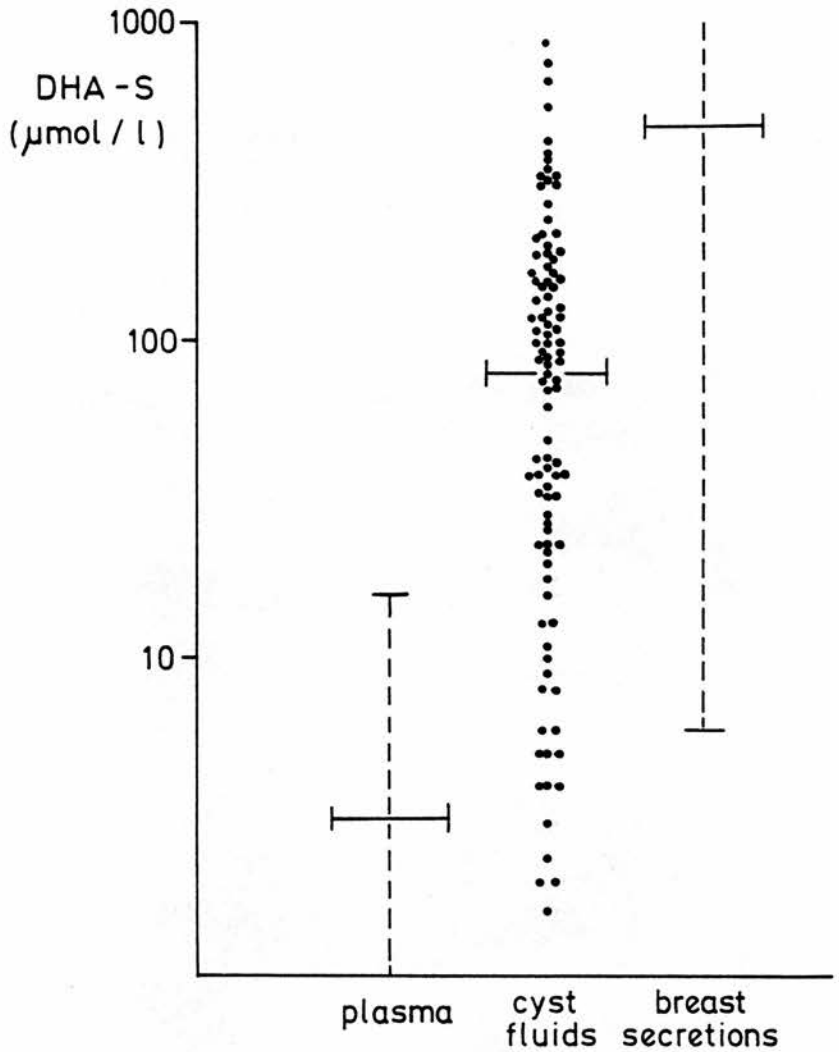


Figure 5 DHA-sulphate concentrations in human breast cyst fluids. Dotted vertical lines represent range in human plasma and breast secretions obtained by nipple aspiration. Horizontal lines represent median values.

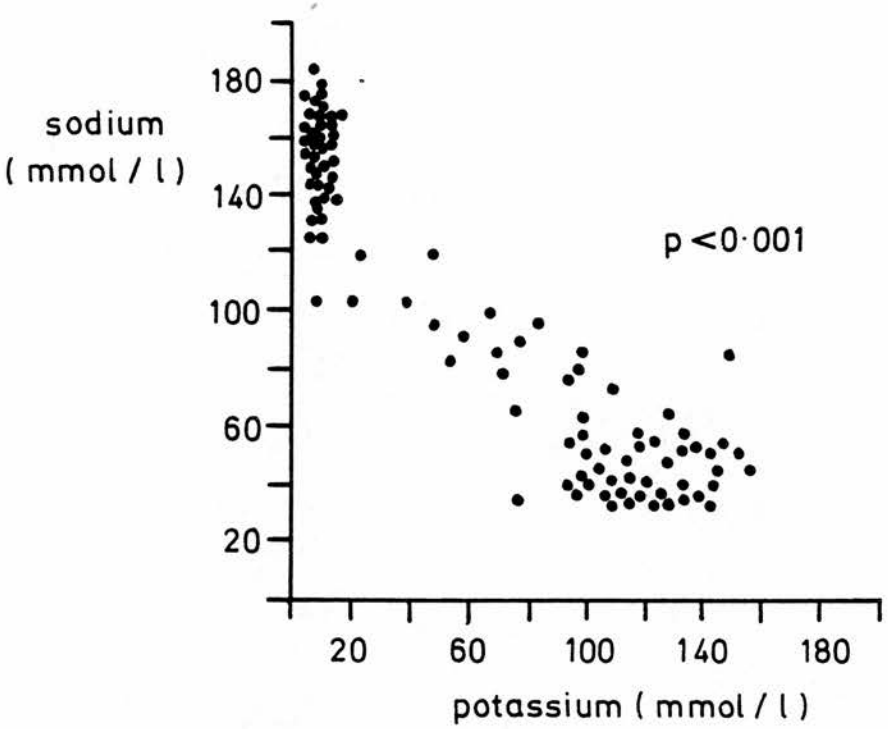


Figure 6 Relationship in human breast cyst fluid between Na+ and K+.

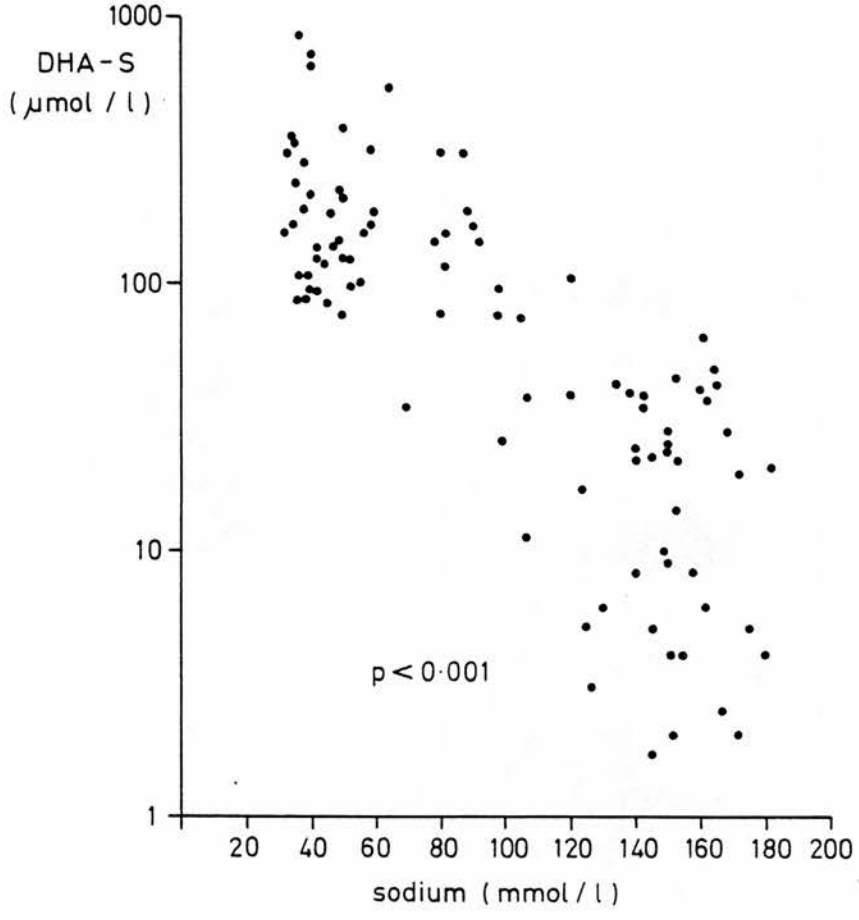


Figure 7 Relationship in human breast cyst fluid between DHA sulphate and Na+.

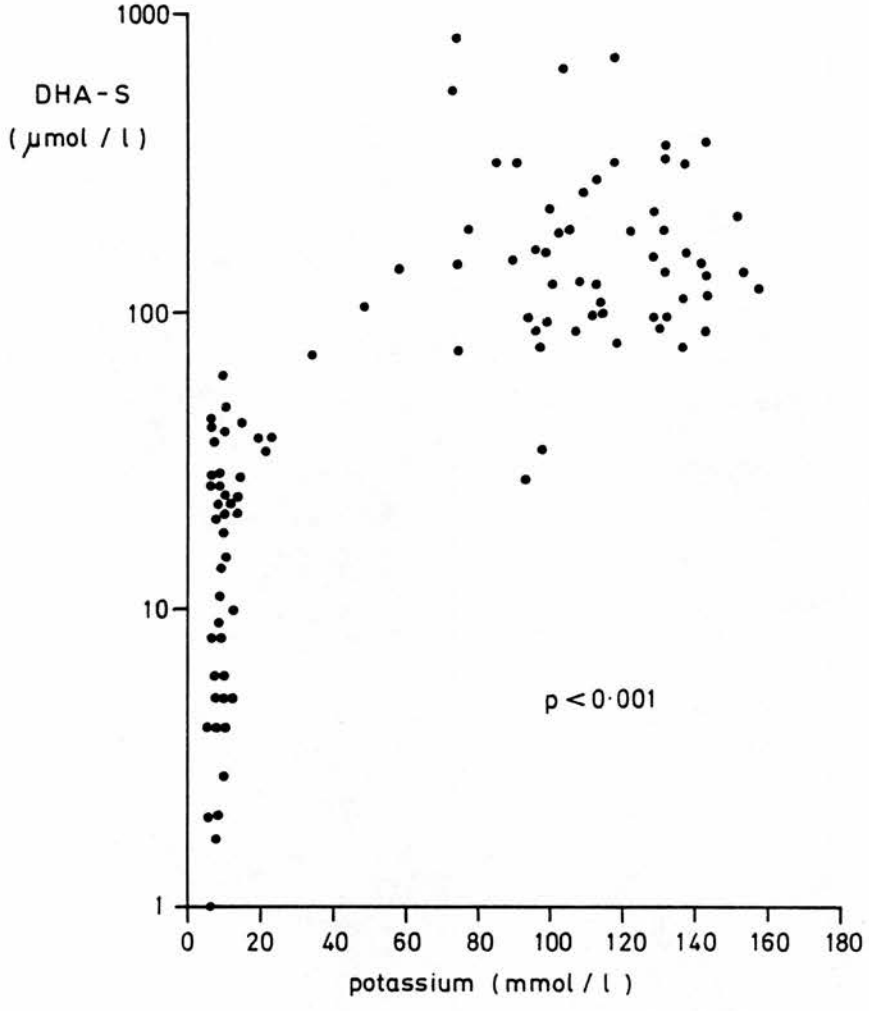


Figure 8 Relationship in human breast cyst fluid between DHA sulphate and K+.

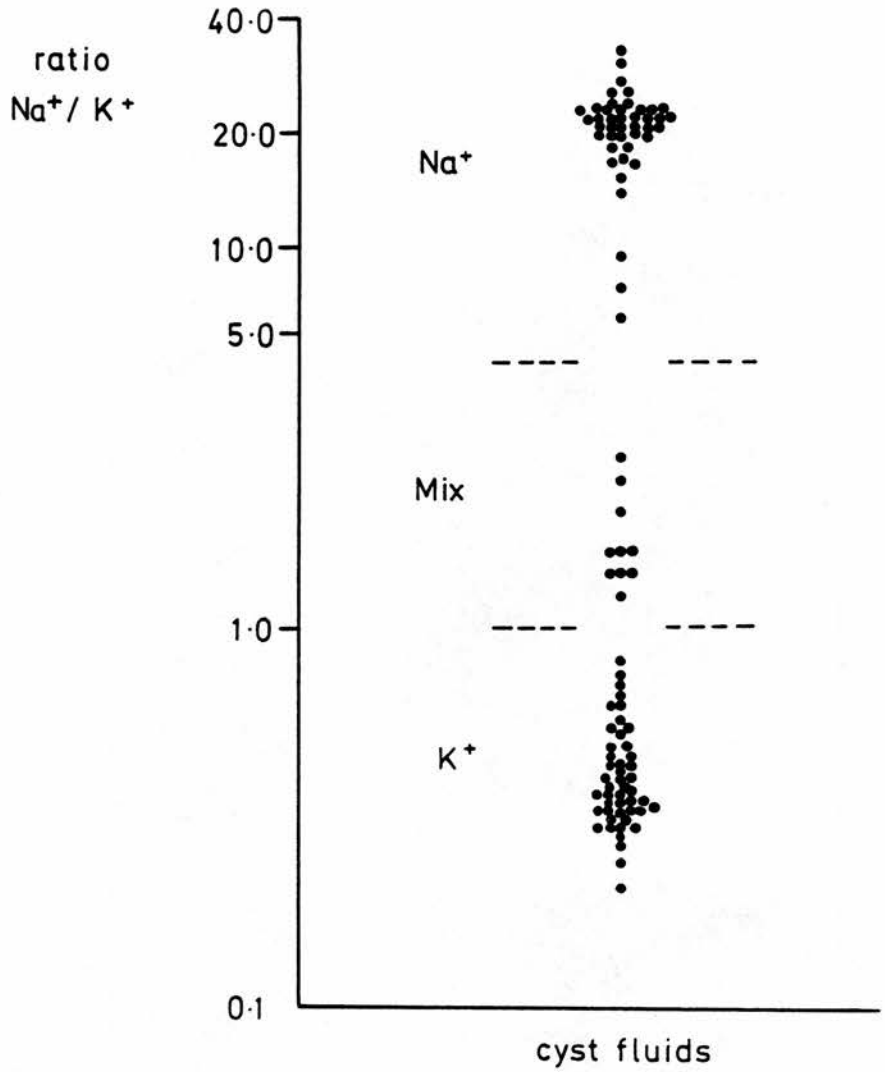


Figure 9 Separation of human breast cyst fluids into 3 groups based on Na+/K+ ratio. Lines represent arbritrary division to give Na+, Mix and K+ fluids.

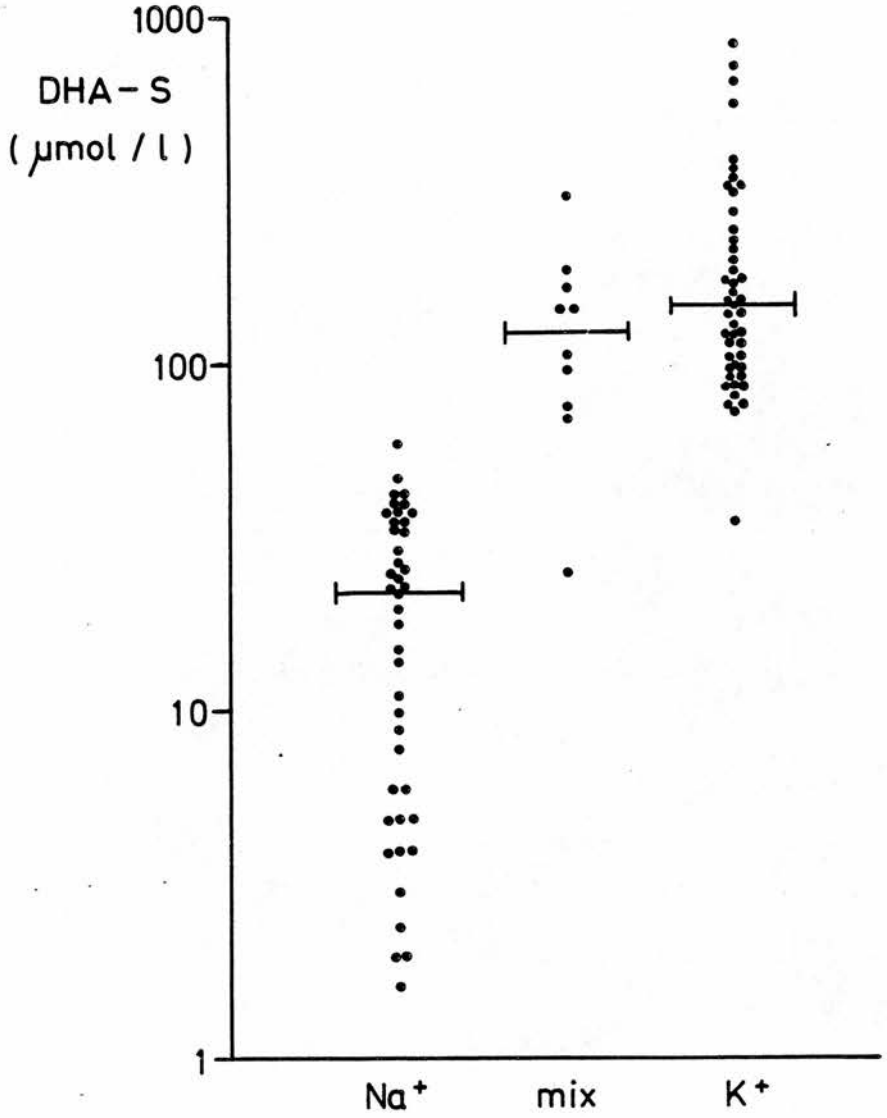


Figure 10 DHA sulphate concentrations in human breast cyst fluids subdivided according to electrolyte classification into 3 groups : Na+, Mix and K+ fluids.

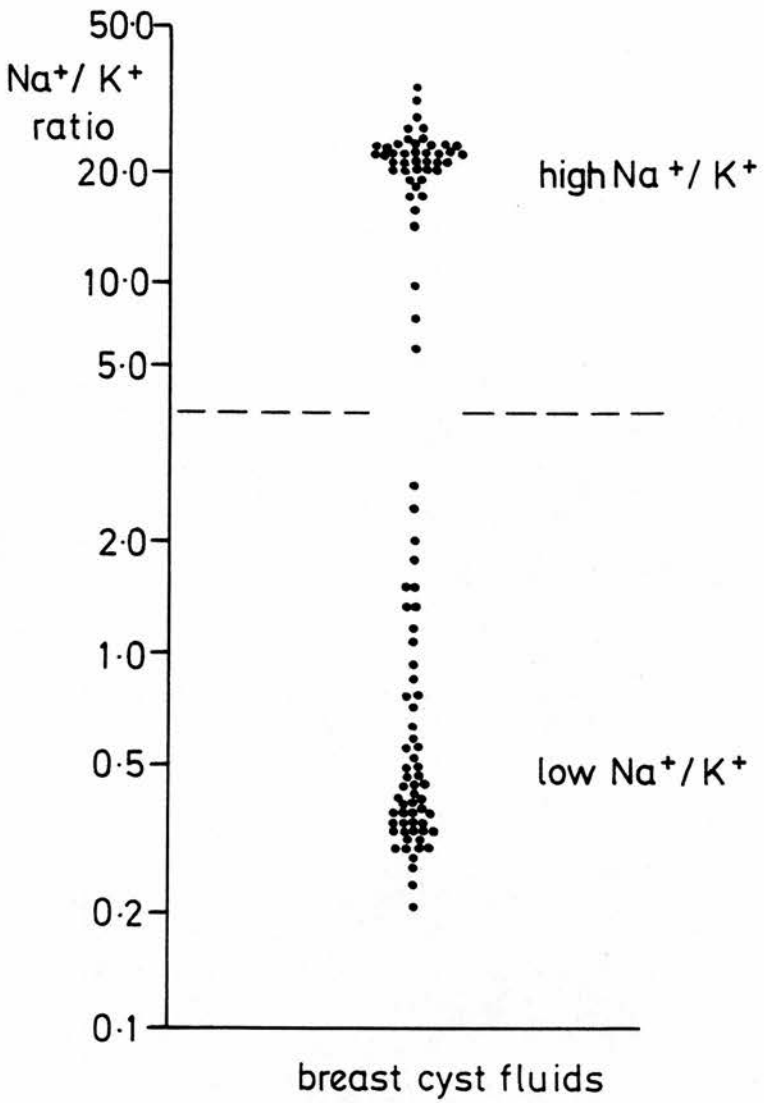


Figure 11 Separation of human breast cyst fluids into those with a high and those with a low Na+/K+ ratio.

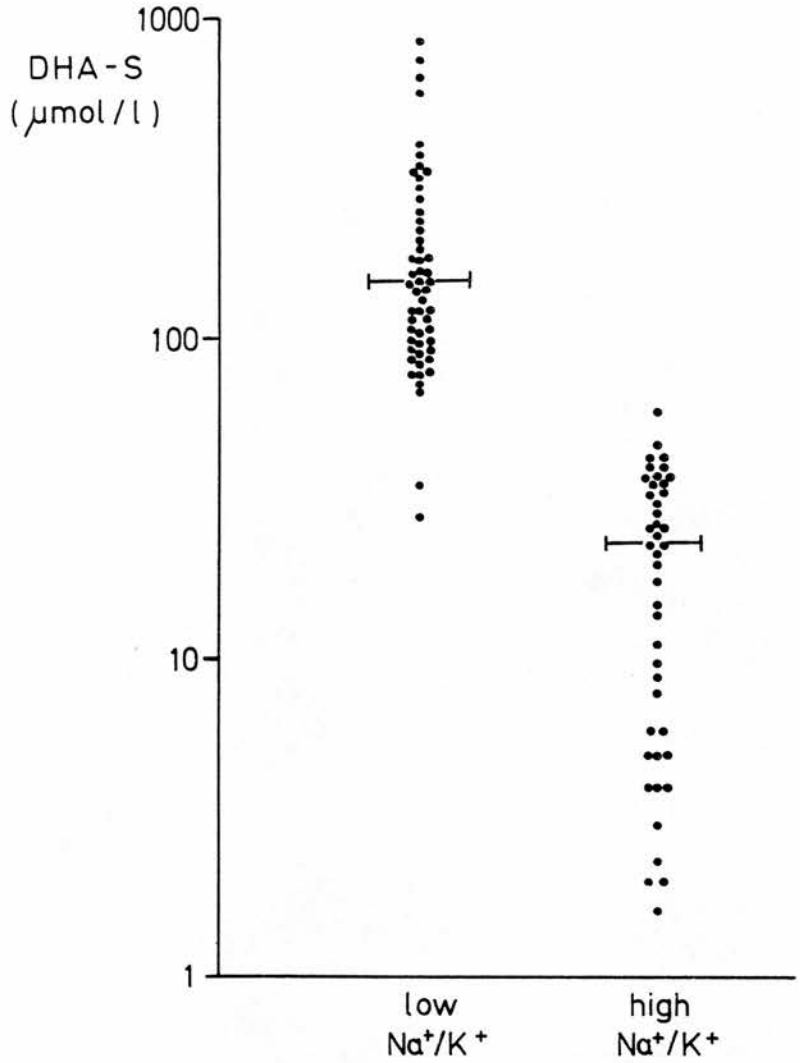


Figure 12 DHA sulphate concentrations in the two populations of human breast cyst fluids defined on the Na+/K+ ratio.

(ii) Proteins and form of IgA in breast cyst fluid

Cyst fluids have been shown to contain IgA in either the secretory (11S) or non-secretory (7S) form and it is possible to subdivide breast cysts into two groups on this basis (Yap et al 1982, Yap et al 1984). Albumin, lactoferrin and IgG were also measured in the same cyst fluids and shown to vary between individual cyst fluids. Significant differences in concentrations of some of these substances were apparent in cyst fluids classified according to IgA type (Yap et al 1982, Yap et al 1984). The fluids upon which these measurements were performed were still available in this department. The aim of this study was therefore to determine if classification by IgA type was the same as that by electrolyte content and also to compare the levels of albumin, lactoferrin and IgG in the two populations of cyst fluids defined by Na^+/K^+ ratio.

Patients, Materials and Methods

Ninety-six fluids aspirated from 75 patients had concentrations of IgG, albumin and lactoferrin measured by immunodiffusion and IgA and DHA sulphate content estimated by radioimmunoassay. The IgG, albumin, lactoferrin and IgA were measured by Dr P L Yap as part of work for a PhD thesis (Yap 1981). These methods, therefore, will not be presented. In the 19 cyst fluids with the highest concentrations of IgA, the form of IgA (11S or 7S) was determined by

sucrose density ultracentrifugation and Na+ and Ka+ were measured by flame photometry. Results presented will be for this group of fluids.

Statistical analysis was by the Wilcoxon Rank sum test or Fisher's exact test.

Results

There were 9 cyst fluids with a low Na+/K+ ratio and 10 cyst fluids with a high Na+/K+ ratio. The concentrations of DHA sulphate IgG, albumin and lactoferrin (Figure 13) were significantly different in the two groups (all $p < 0.01$, except lactoferrin $p < 0.05$). Concentrations of IgA were similar in both groups (Figure 14) but the predominant form in the cysts with a low Na+/K+ ratio was the secretory (11S) form, in contrast to the group with a high Na+/K+ ratio where the 7S form predominated (Table I). This correlation was significant ($p = 0.0001$). Comparison of IgA type and DHA sulphate concentration is shown in Figure 15 and demonstrates that the one cyst fluid with a high Na+/K+ ratio which had predominantly 11S IgA also had a low concentration of DHA sulphate.

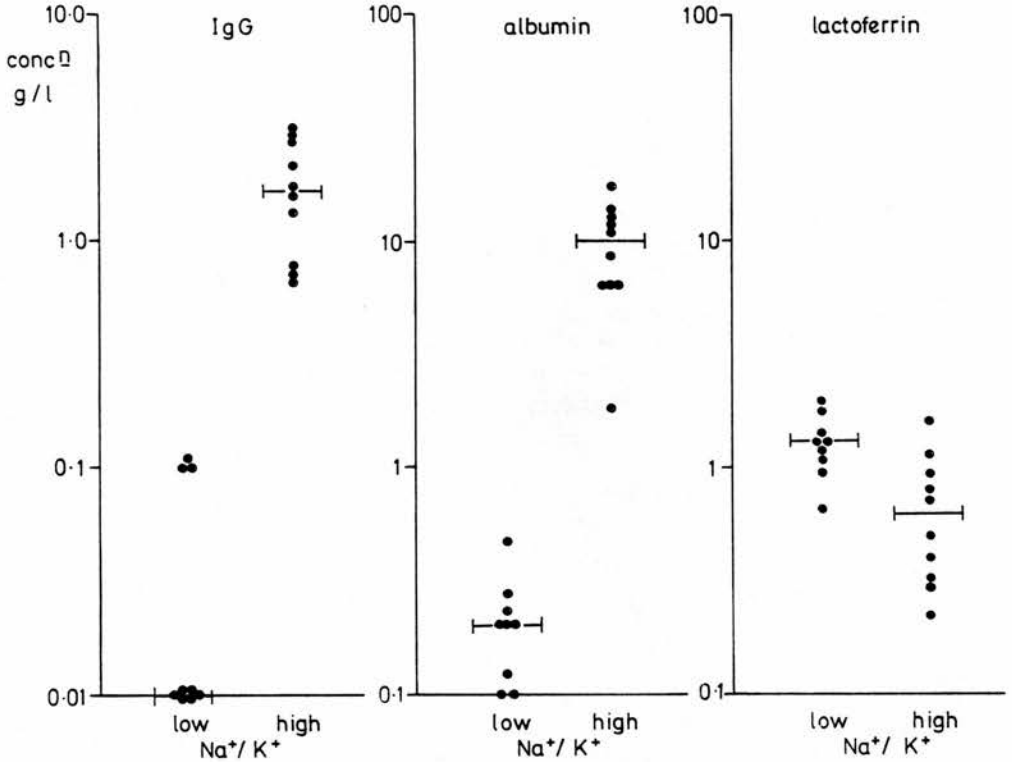


Figure 13 Concentrations of IgG, albumin and lactoferrin in cyst fluids subdivided on the basis of the Na+/K+ ratio.

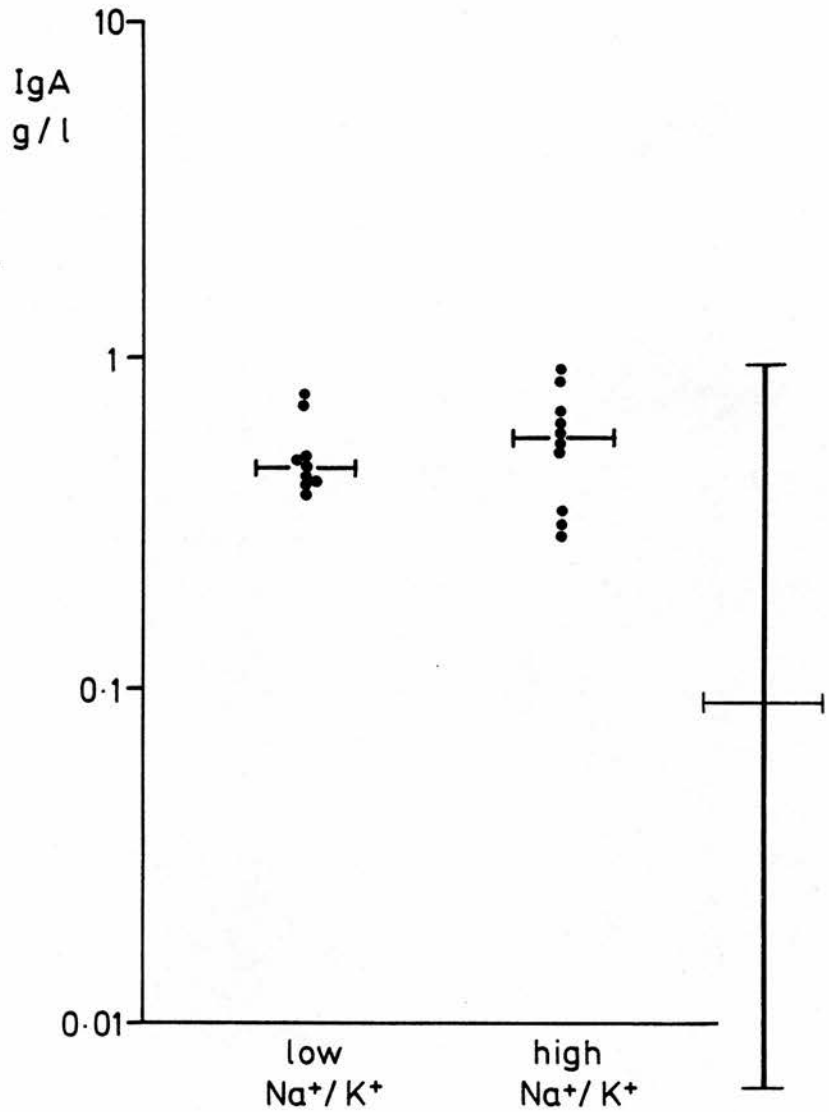


Figure 14 Concentrations of IgA in cyst fluids subdivided on the basis of the Na+/K+ ratio. Normal range of IgA in plasma shown as solid vertical line (horizontal bars represent median values)

		Predominant form of IgA	
		11S	7S
Na+/K+ ratio	low	9	0
	high	1	9

Table I Relationship between IgA type and electrolyte classification in human breast cyst fluids.

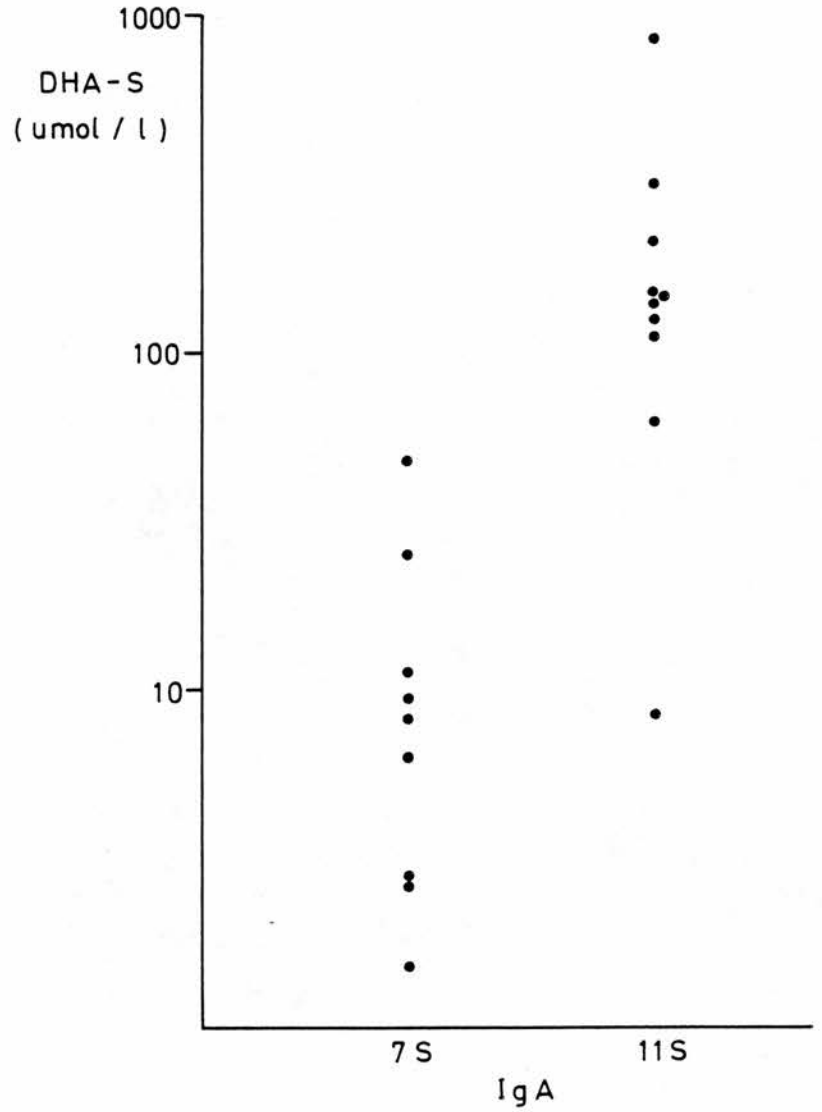


Figure 15 Concentrations of DHA sulphate in cyst fluids subdivided on the basis of the predominant type of IgA (7s or 11s).

(iii) pH of Breast Cyst Fluids

The aim of the present study was to measure pH in fresh cyst fluids to determine if differences in pH exist between the two populations of cyst fluids defined on the ratio of Na^+ to K^+, and also to determine the effect of storage of fluids on pH.

Patients, Materials and Methods

One hundred and six breast cyst fluids were aspirated from 74 patients into airtight syringes, placed on ice and pH measured within 1 hour using a Corning 178 pH/blood gas analyser. Na^+ and K^+ concentrations were measured in the same fluids by flame photometry. Ten fluids were stored at -20°C and pH was remeasured 1 week and 1 month after aspiration.

Forty-four cysts had pH estimated using Multistix reagent strips (Ames Division, Miles Laboratories, Slough, England).

Statistical correlation of pH and Na^+/K^+ ratio was by the Kendall Rank test and comparison of pH in the two populations of cysts defined by Na^+/K^+ ratio was made using the Wilcoxon Rank sum test.

Results

The pH values of the 106 cyst fluids ranged from 6.3 to 7.8 (Figure 16). There was a significant positive correlation between pH and Na^+/K^+ ratio, $p < 0.001$ (Figure 17). Comparison of fluids subdivided into different types according to Na^+/K^+ ratio (cut off $Na^+/K^+ < 3$ or ≥ 3) showed no overlap in values between the two populations, the difference between the groups being statistically significant ($p < 0.001$) (Figure 18).

The effect of storage on pH in 10 cyst fluids is shown in Figure19. After 1 week pH had increased in all fluids by a mean of 0.4 and by 1 month it had further increased by a mean of 0.9 of a pH unit over initial values.

pH as estimated by Multistix also differed in the two groups of fluids defined on electrolyte content (Table II). It is thus possible to separate cyst fluids into two groups by a simple assesment of pH with these strip reagents.

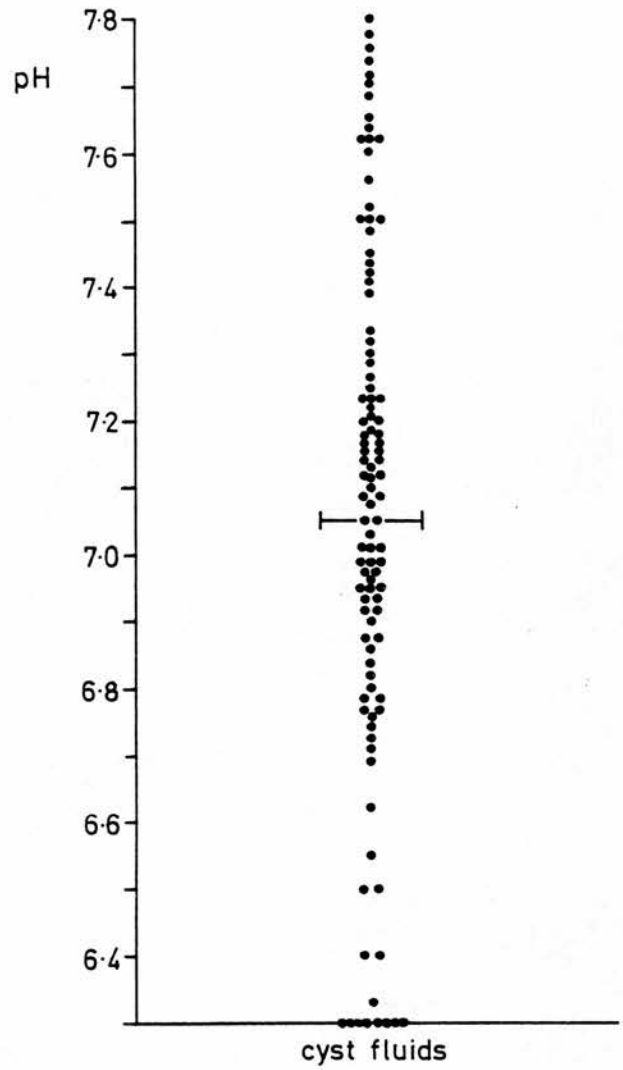


Figure 16 pH of human breast cyst fluids.

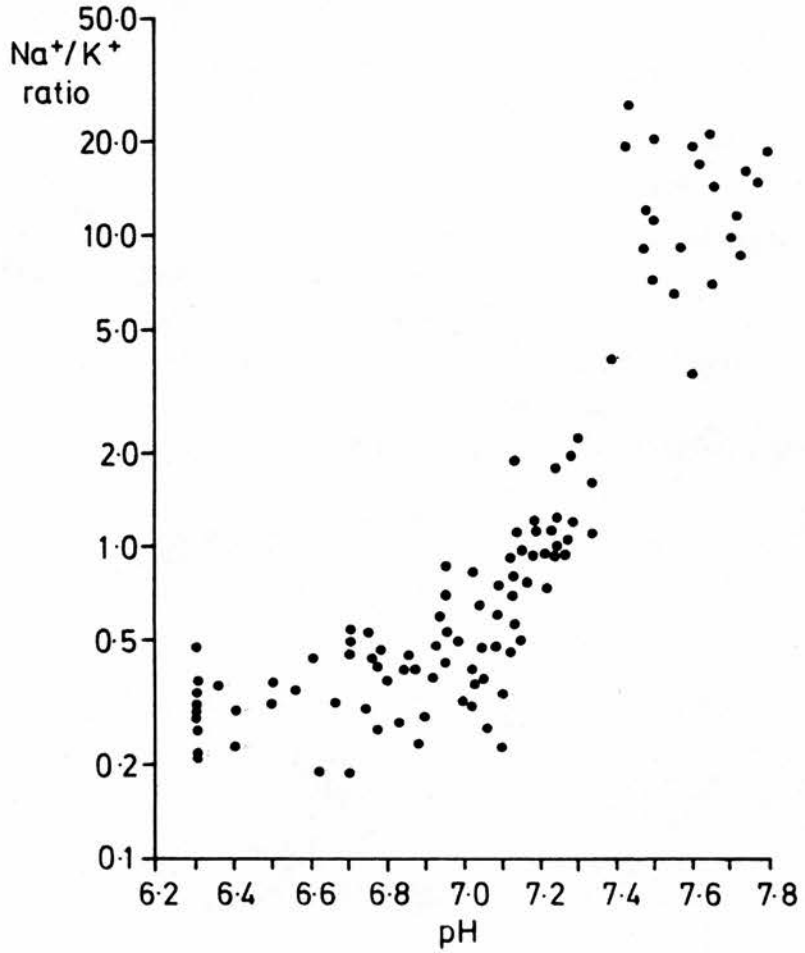


Figure 17 Relationship between pH and Na+/K+ ratio in human breast cyst fluids.

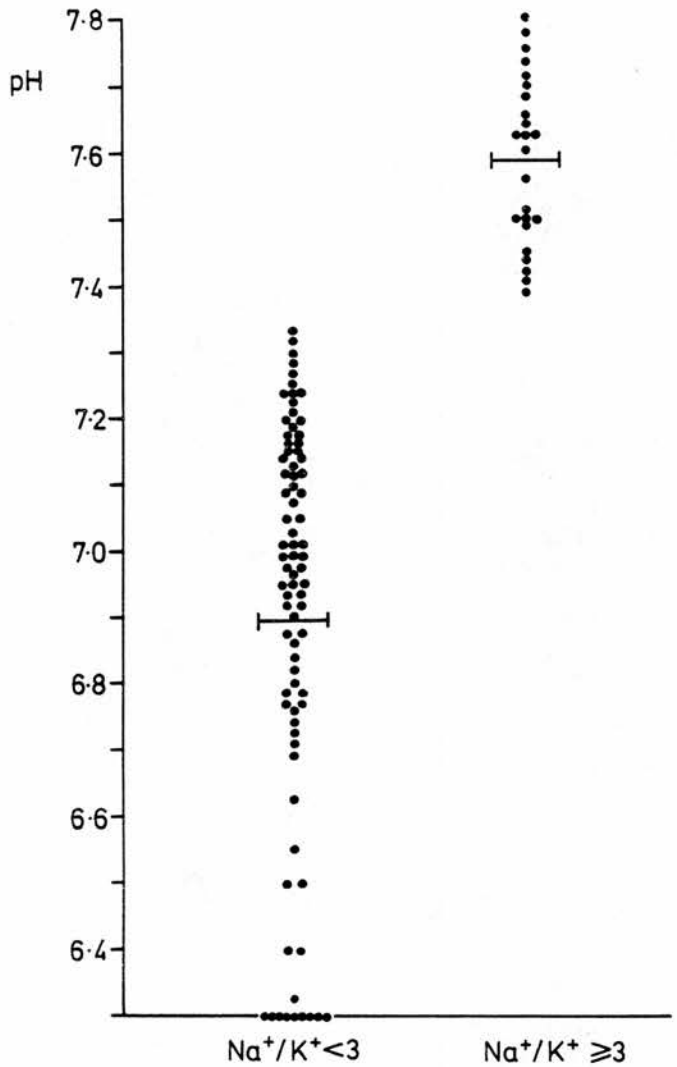


Figure 18 pH values in the two populations of cyst fluids defined on the basis of Na+/K+ ratio.

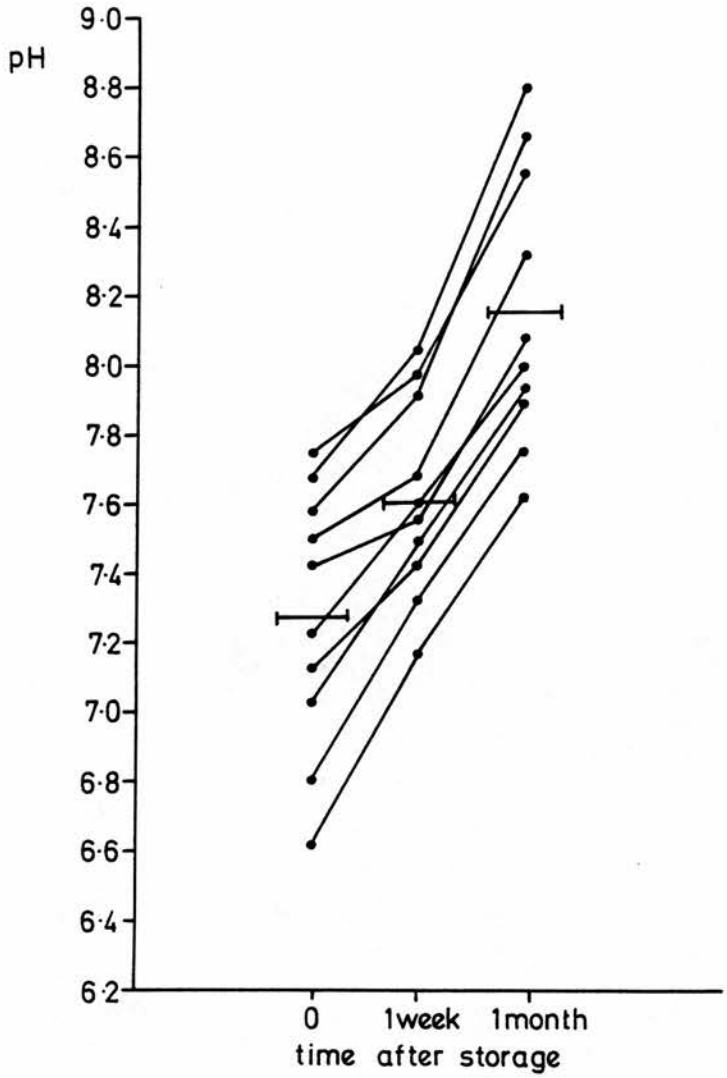


Figure 19 Effect of storage of cyst fluids on pH. Horizontal bars represent mean values.

	number of cysts with low Na+/K+ ratio	
	low (n = 28)	high (n = 16)
pH		
6.0	2	-
6.5	21	-
7.0	5	-
7.5	-	14
8.0	-	2

Table II Comparison of pH as assessed by Multistix in the two populations of cyst fluids defined on the basis of Na+/K+ ratio in 44 cyst fluids.

(iv) Factors affecting the composition of breast cyst fluid

The aim of the present study was to determine which factors might influence the concentrations of Na+, K+ and DHA sulphate in breast cyst fluids. The factors studied included age of the patient, menopausal status, parity, volume of the cyst, whether cysts were single or multiple, whether the patient was taking the oral contraceptive pill, and length of time the cyst had been present.

a) Age, Menopausal Status and Parity

The concentration of DHA sulphate in cyst fluid measured by radioimmunoassay was compared with age and menopausal status in 40 patients who had 46 cysts aspirated and is shown in Figures 20 and 21. Age did not relate to DHA sulphate. Median DHAS concentrations tended to be lower in the cysts of postmenopausal women but the differences in the two groups did not reach statistical significance. Parity similarly showed no significant correlation to DHA sulphate content of cyst fluid.

b) Volume

A comparison of DHA sulphate concentrations and cyst volume for the 100 patients studied for Na+, K+ and DHA sulphate is shown in Figure 22. There was no significant association between these two parameters.

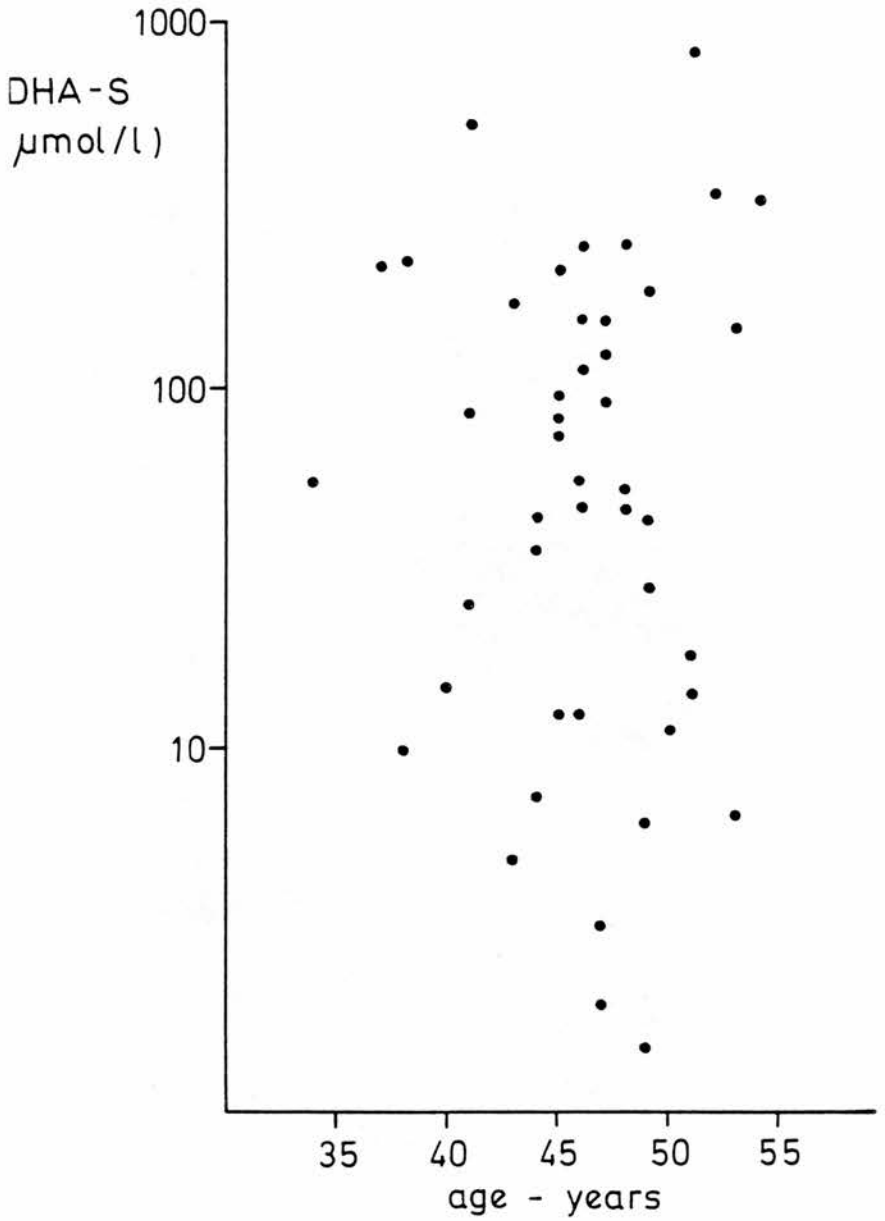


Figure 20 Comparison of DHA sulphate concentrations in human breast cyst fluids with age of the patient.

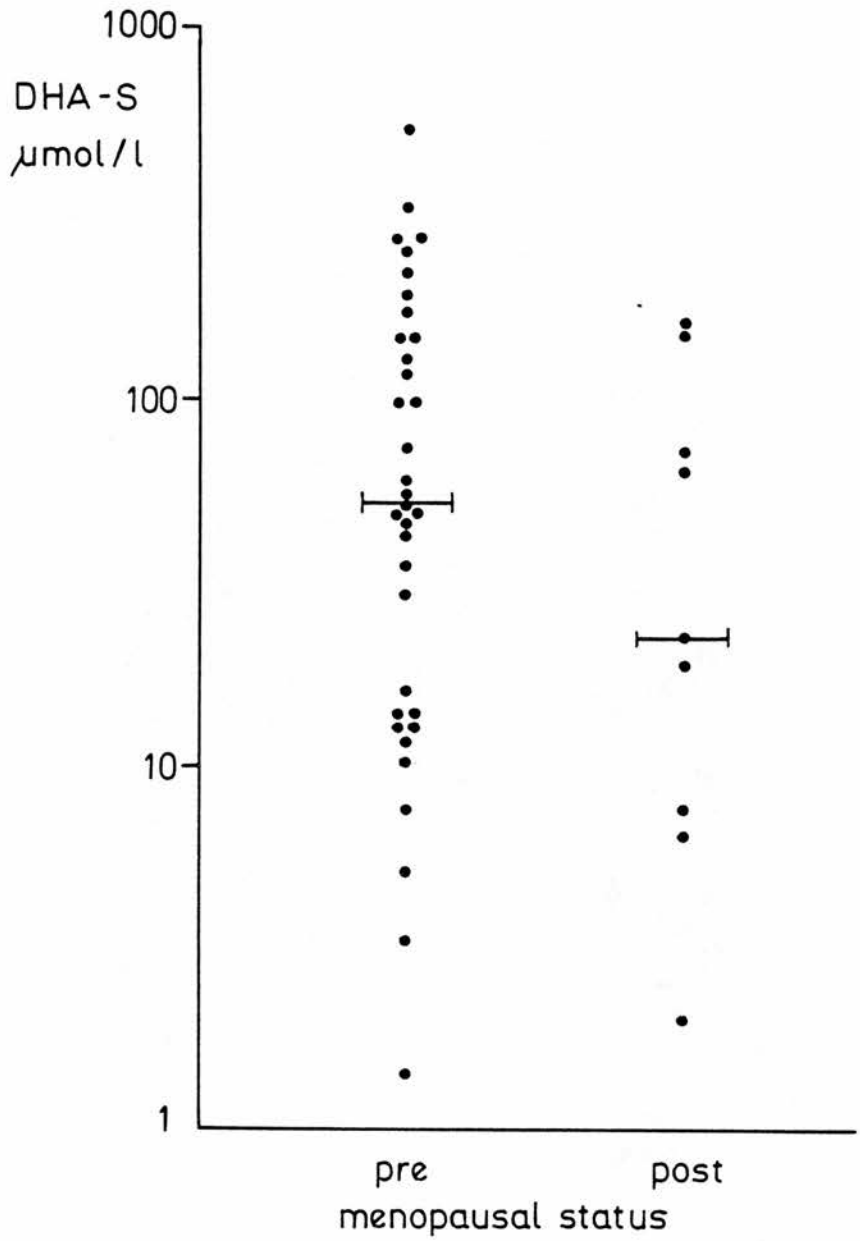


Figure 21 Comparison of DHA sulphate concentrations in human breast cyst fluids with menopausal status of the patient. Pre-menopausal was defined as women who had a period within last 3 months. Postmenopausal defined as women whose last menstrual period was greater than 3 months before cyst operation.

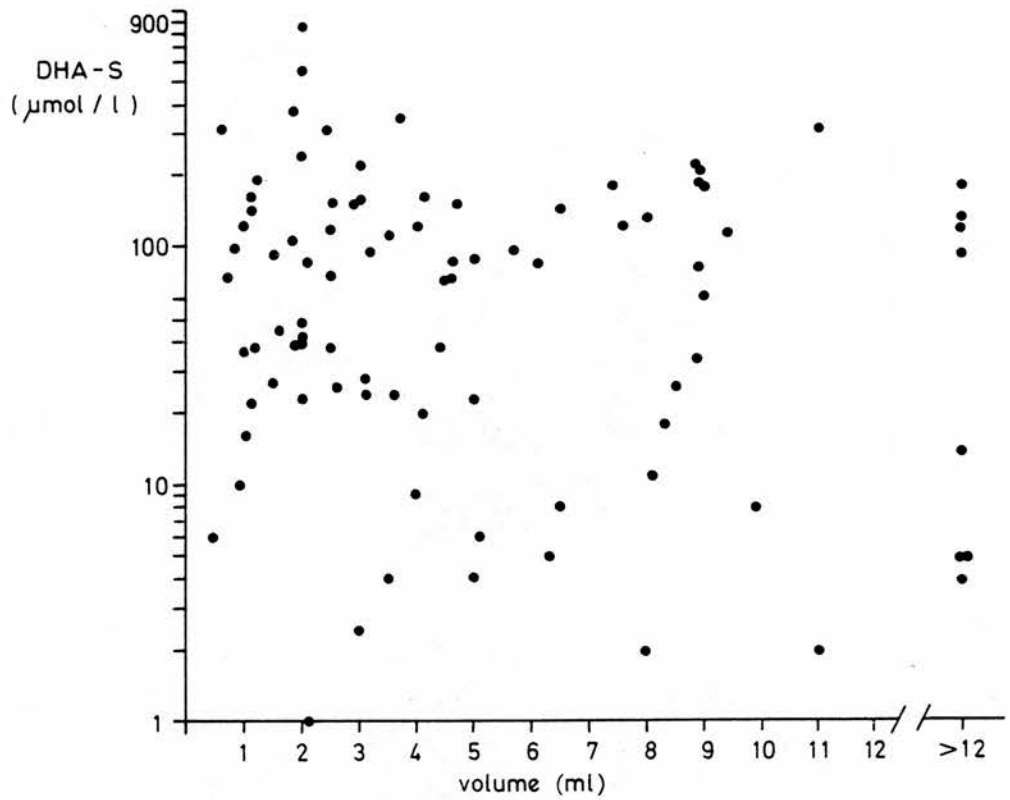


Figure 22 Comparison of DHA sulphate concentrations in human breast cyst fluids with volume of the fluid aspirated.

c) Multiplicity

In the group of 85 patients with 100 cysts, 10 patients had multiple cysts in one or both breasts. Table III gives details of these 10 patients with the cysts classified on the basis of the Na+/K+ ratio in cyst fluid. It is evident that in nine of the 10 patients, the cysts each patient developed were all of the same type, ie they all had either a low or a high Na+/K+ ratio. One patient had mixtures of cysts. Seven of the 9 patients whose cysts were all the same type had cysts with a low Na+/K+ ratio and two had had cysts with a high ratio. Comparing this with the overall distribution of cysts (57% low ratio) multiple cysts appear more likely to have a low ratio and thus a high DHA sulphate (in multiple cysts 78% low ratio).

	Breast 1			Breast 2
Patient	Cyst 1	Cyst 2	Cyst 3	Cyst 1
1	high	high		
2	high	high	high	
3	low	low		
4	low	low		
5	low	low		
6	low	low	low	
7	low	low	low	low
8	low			low
9	low			low
10	high	low		high

Table III Ten patients with multiple cysts with cysts classified according to ratio of Na^+ to K^+.

d) Oral contraceptives

A review of all patients who had cyst fluids stored on file within this department identified only 13 patients who were taking the combined oral contraceptive pill at the time they had their breast cyst aspirated. All 13 patients had been on the contraceptive pill for at least 6 months. From the data on patients who had either previously taken the pill (on pill for at least 6 months, last taken pill >12 months prior to cyst aspiration) or who had never been on the pill, 2 groups of 13 patients were matched with the current pill takers for age (within 2 years) menopausal status and parity (+1).

All cyst fluids aspirated from these 39 patients had Na+ and K+ estimated and the groups were then compared using the X^2 test.

There were 16 cysts aspirated in the 13 patients in the group on the pill, 55 cysts aspirated in the group previously on the pill and 39 cysts in the group who had never taken the pill. There were significantly larger numbers of cysts in both the patients previously on the pill and those never on the pill ($p < 0.01$ for both). A summary of the Na+/K+ ratio in these cyst fluids is shown in Table IV. It shows a significantly lower percentage of cyst fluids with a low Na+/K+ ratio in the group taking the pill at the time of cyst aspiration ($p < 0.001$ compared with both groups) and a significant reduction in the number of patients with these cysts when compared to the other two groups combined ($p < 0.05$).

	Presently on Pill	Previously on Pill	Never on Pill
Number of patients	13	13	13
Number of cysts	16	55	39
Number of cysts with low Na+/K+ ratio	7 (44%)	49 (89%)	35 (90%)
high Na+/K+ ratio	9	6	4
Number of patients with any cysts having a low Na+/K+ ratio	4 (31%)	9 (69%)	9 (69%)

Table IV Cyst fluids subdivided into two populations on Na+/K+ ratio in the matched groups of: patients presently on the pill; patients previously on the pill and those who had never taken the pill.

e) Length of history and cyst composition

It has been suggested that the cyst epithelium becomes flattened as time since formation increases and that the colour of cyst fluid correlates with age of the cyst (Haagensen et al 1981). A prospective study comparing length of history of the breast mass, electrolyte composition and cyst fluid colour was undertaken in 75 cysts aspirated from 50 patients.

Cysts were separated into three groups on the basis of colour(straw or yellow, green and blue/black). Comparison of composition and length of history in weeks were made between these 3 groups.

There was no correlation between length of history and composition, length of history and colour or colour and composition. This indicates that neither composition; nor colour are related to the length of time which a cyst has been present.

(v) Correlation of the concentration of DHA sulphate in breast cyst fluid and breast secretions in the same patient

Introduction

Having identified that the concentrations of DHA sulphate vary widely in breast cyst fluids and breast secretions obtained by nipple aspiration, the aim of the present study was to determine if there was a correlation between values in individual women.

Patients, Materials and Methods

Breast secretions were obtained from one or both breasts in 10 patients on the same day they had a single breast cyst aspirated. DHA sulphate was estimated in samples of cyst fluid and breast secretions by radioimmunoassay. Na^+ and K^+ were also measured in cyst fluid by flame photometry. The Kendall rank test was used to compare DHA sulphate values in the two fluids. The Wilcoxon rank sum test was used to compare DHA sulphate levels in breast secretions from patients who had cyst fluids with a low or high Na^+/K^+ ratio.

Results

There were 18 samples of breast secretions and 10 cyst fluids aspirated from the ten patients. Figure 23 shows a comparison of DHA sulphate concentrations in cyst fluids and breast secretions. The correlation coefficient for this comparison was -0.22, indicating no significant correlation between values of DHA sulphate in those two fluids when aspirated on the same day from individual patients.

Six cyst fluids had a low Na^+/K^+ ratio and four had a high Na^+/K^+ ratio. DHA sulphate levels in breast secretions in the two groups subdivided on Na^+/K^+ ratio in cyst fluid are shown in Figure 24. There was no significant difference in DHA sulphate concentrations in the two groups.

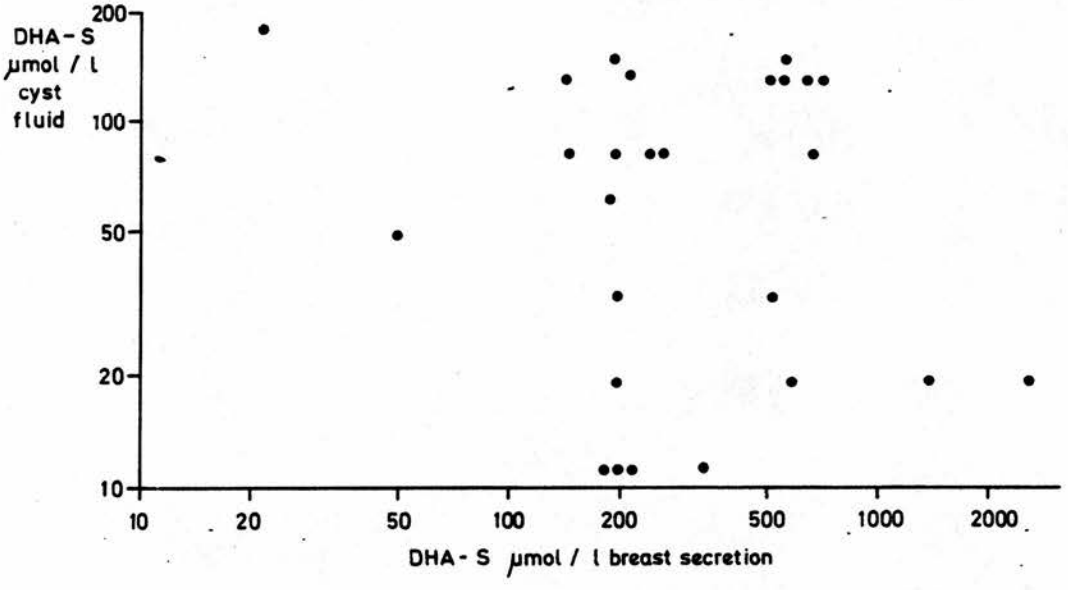
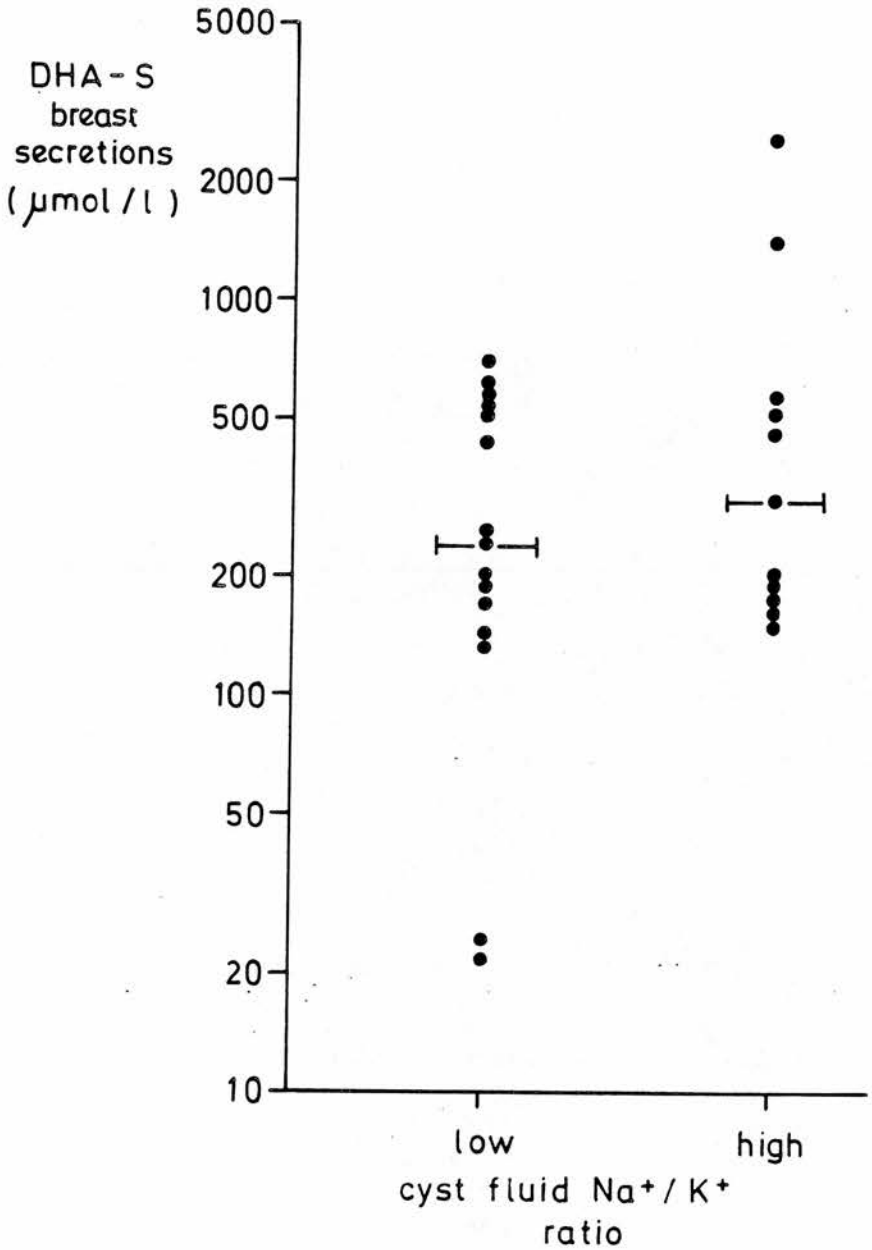


Figure 23 Comparison of DHA sulphate concentrations in cyst fluids and breast secretions aspirated from same patients.



.Figure 24 Concentration of DHA sulphate in the breast secretions of patients with breast cysts subdivided according to the Na+/K+ ratio in the aspirated cyst fluid

(vi) Correlation of composition of cyst fluid and frequency and volume of breast secretions

Introduction

Cysts with a low Na+/K+ ratio contain active secretory products (11S IgA and lactoferrin) and it may be that they arise in breasts with a greater degree of secretory activity. The aim of the present study was to compare the frequency with which breast secretions are obtained from women with cysts having either a high or low Na+/K+ ratio and to compare the volumes obtained in the two groups.

Patients, Materials and Methods

From data on file for over 1,000 women, from whom it has been attempted to obtain breast secretions, two groups of women were identified: 21 non-secretors and 32 secretors who had a history of cyst aspiration on the same date that an attempt was made to obtain secretions by nipple aspiration. Na+ and K+ were estimated in all cyst fluids from these patients by flame photometry and then a comparison of the frequency and volumes of secretion obtained in women with the two types of cyst performed using the X^2 and Wilcoxon rank sum tests respectively.

Results

Eighteen non-secretors and 30 secretors had cysts aspirated which were all of one or other type, ie all cysts had a low or high Na+/K+ ratio. The remaining 5 patients had mixtures of the two cyst types. Overall, 19 had cysts with low Na/K+ ratios and from 11 secretions were obtained (58%); 29 had cysts with high Na+/K+ ratios and secretions were obtained in 19 (66%). There was no significant difference in the frequency of obtaining breast secretions from either group.

From the 11 secretors with cysts having a low Na+/K+ ratio, secretions were obtained from both breasts in 9 (82%). In contrast, secretions were obtained from both breasts in only 8 of the 19 (42%) women whose cysts had a high Na+/K+ ratio. This difference was significant, $p < 0.05$.

The total volume of secretion from both breasts in each patient was calculated and then the volumes of secretion in women whose cysts had either high or low Na+/K+ ratios compared as shown in Figure 25. The volumes of secretion in the group of patients with cysts with a low ratio were significantly higher (median volume 12.8 µl) than in patients with cysts having a low Na+/K+ ratio (median volume 4.4 µl), $p < 0.02$.

Although patients with active secretory cysts (11S IgA low Na^+/K^+ ratio) are not associated with an increased frequency in obtaining breast secretions, they are associated with a greater likelihood of obtaining secretions from both breasts and an increased volume of breast secretions.

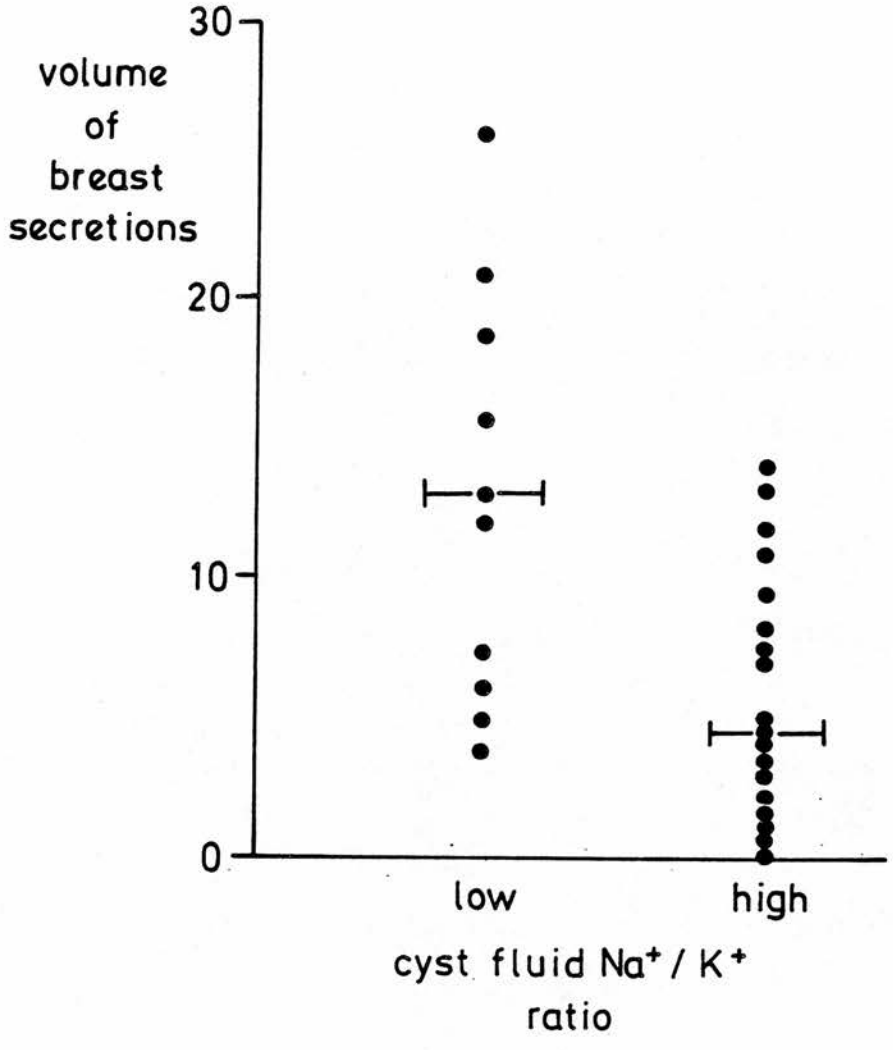


Figure 25 Volumes of breast secretions obtained from patients with breast cysts subdivided according to the Na+/K+ ratio in the aspirated cyst fluid

DISCUSSION

These studies confirm the wide variation in levels of Na+, K+ and DHA sulphate in breast cyst fluids (Bradlow et al 1981a, Bradlow et al 1981b, Miller et al 1982, Bradlow et al 1983a, Bradlow et al 1983b, Miller et al 1983). They further show significant associations between the concentrations of these three constituents. As has been previously noted, the levels of Na+ and Ka+ in cyst fluids are not normally distributed around their mean values, suggesting that there may be more than one population of cyst fluids (Bradlow et al 1981b). Using the relative concentrations of Na+ and K+, it has been possible to define two populations of cyst fluids, a classification which also subdivides cyst fluids in those with high or low levels of DHA sulphate.

Cyst fluids have been previously classified into two groups on the basis of the predominant form of IgA present in the fluid (Yap et al 1982). A comparison of 19 cyst fluids subdivided on the basis of Na+/K+ ratio was almost identical to that based on the predominant form of IgA. Cyst fluids with a low Na+/K+ ratio were shown to have predominantly (secretory) 11S IgA, whereas those with a high ratio had 7S (non-secretory) IgA. There was, however, one fluid which differed in the two classifications, a cyst with a high Na+/K+ ratio which had predominantly secretory IgA. This contained low levels of DHA sulphate, indicating that the cyst fluid was more likely to be of non-secretory type, suggesting that it was correctly classified on the basis of electrolyte composition but not IgA type. Cyst fluids

with a low Na+/K+ ratio in addition to having secretory IgA, also contain higher concentrations of a further secretory product lactoferrin, whereas cysts with a high Na+/K+ ratio contain the 7S form of IgA and greater amounts of albumin and IgG and thus more closely resemble the composition of plasma.

Human breast cyst fluids have been previously reported to be alkaline, with a pH range of 7.6 - 9.0 (Gatsy et al 1979). These measurements were, however, performed on stored samples and this study has shown that fresh cyst fluids are acidic or neutral and only become alkaline on storage. This finding of a change in pH may have more important implications when interpreting data on enzymatic activity in stored cyst fluid, as activity is known to change with pH (Schwartz et al 1976).

Having identified two populations of cyst fluids, it remains to establish how these two fluids are derived. There exists two possibilities:

(i) there may be differences in the secretory activity of the epithelium from which these fluids are derived, or

(ii) the fluids in the two groups of cysts may be derived from different sources, namely plasma and breast.

The composition of cyst fluids with a high Na+/K+ ratio is similar to that of plasma (Table V). Although DHA sulphate levels in cyst fluids with a low Na+/K+ ratio are similar to those in breast secretions obtained by nipple aspiration, Na+ is the predominant ion in these secretions (Table V). Also, in any one patient, there was no apparent relationship between DHA sulphate levels in secretions and cyst fluids, even for the fluids which may be breast derived. Simple accumulation of secretions is therefore unlikely to account for the formation of these cyst fluids.

It seems more likely that the two fluids may arise from epithelium with differences in secretory activity. Certain cysts are known to be lined by apocrine secretory epithelium (Azzopardi 1979) and it is known that apocrine secretions from other sites in the body contain high concentrations of DHA sulphate (Labows et al 1979). Apocrine secretion occurs by the expulsion of intracellular secretory granules (Azzopardi 1979). Intracellular fluid has a lower pH than extracellular fluid (Waddell et al 1969) and apocrine secretion from the axilla is known to be acidic (Hurley et al 1960). The findings of active secretory products (11S IgA and lactoferrin) and a low pH in cyst fluids with a low Na+/K+ ratio would thus be consistent with fluids derived from apocrine epithelium. In contrast, those fluids with a high Na+/K+ ratio which have been previously noted to resemble plasma in composition may be derived from a less active epithelium by transudation.

FLUID		Na+/K+	[DHA sulphate]	Predominant form of IgA	IgG	Albumin	Lactoferrin	pH
				COMPOSITION				
CYST FLUID	Group 1	low	↑↑	11S	↓↓	↓↓	↑	acid
	Group 2	high	→	7S	→	→	→	neutral
PLASMA		high	→	7S	→	→	→	neutral
BREAST SECRETIONS		high	↑↑	11S	↓		↑	not known

Table V : Comparison of the composition of the two populations of cyst fluids, plasma and breast secretions obtained by nipple aspiration (→ indicates similar to level in plasma with ↑ an increase and ↑↑ a great increase above plasma levels. Similarly ↓ and ↓↓ signify lower and greatly reduced levels compared to plasma)

It might be expected that the secretory activity of breasts in which active secretory cysts arise might be greater than that of breasts in which inactive cysts arise. The frequency of obtaining breast secretions from both groups of patients were similar, but secretions were more commonly obtained from both breasts and the total volumes of secretions obtained were greater in patients with cysts with a low Na^+/K^+, suggesting that in those women who do produce secretions, greater amounts of secretion may be produced in patients with active secretory cysts.

Neither population of cysts was more frequent in any age group and their occurrence did not differ in pre and postmenopausal women. Volume was also unrelated to composition. This latter finding indicates that an active secretory cyst probably does not become less active when it becomes larger. The observation that there is no correlation between the length of time a cyst has been present and composition also suggests that there is no transition from one type of cyst to another. Further support for this comes from the finding that multiple simultaneous cysts in any one woman tend to be of the same type. Thus, there may be a factor which determines whether a patient develops cysts of one or other type. The patients with multiple cysts were also noted to have a higher frequency of cysts with low Na^+/K^+ ratios. It may be then that the composition of cyst fluids relates to subsequent behaviour and the natural history of cystic disease.

Long-term use of the oral contraceptives has been reported to be associated with a diminished occurrence of cystic breast disease (Vessey et al 1972, Boston Collaborative Drug Surveillance Programme 1973, Sartwell et al 1973, Kelsey et al 1974, Fasal et al 1975, Ory et al 1976, Cole 1977, Royal College of General Practitioners 1977, Lee et al 1978, Pastides et al 1983). Only 13 women from a series of over 600 were identified in the present study who were taking oral contraceptives at the time they developed a cyst. These women had significantly less cysts than matched groups of women who had either previously taken the oral contraceptive or had never taken it. This supports the finding in biopsies of women taking the contraceptive pill which have shown less gross cysts in these subjects (Pastides et al 1983). There was no reduction in the number of cysts aspirated from women who had previously taken the pill. It has been reported that the protective effect of the oral contraceptive agent in reducing benign breast disease carries on for many years after use (Cole 1977). It may be, however, that the length of time which had passed since their use was too great to see this effect in the present study.

The cysts in women on the pill appear to be less likely to contain fluid with a low Na^+/K^+ ratio. If, as has been proposed, this population of cysts is derived from apocrine epithelium, then it is of relevance (i) that the oral contraceptive is known to reduce secretion from the apocrine ceruminal glands of the external ear (Royal College of General Practitioners 1974) and (ii) that the pill has also been shown to reduce the frequency of apocrine change in

the breast as assessed by breast biopsy (Pastides et al 1983). In this department it has also been shown that, contrary to the initial studies of Petrakis et al (1975), the pill reduces the frequency of fluid obtained by nipple aspiration from non-pregnant women. The contraceptive pill may therefore reduce the secretory activity of the breast with a resultant decrease in the frequency of active secretory cysts.

In summary, two populations of cyst fluids can be defined on the basis of composition and these may be derived from different types of epithelium. The oral contraceptive reduces the number of cysts in patients, probably by reducing secretory activity in the breast The clinical relevance of these two populations remains to be investigated.

Studies on breast cyst epithelium

(i) The relationship between epithelial lining and composition of breast cysts

(ii) Electron microscopy studies of breast cyst epithelium

(iii) Peroxidase localisation of DHA sulphate in breast cyst epithelium

The relationship between epithelial lining and composition of breast cysts

Introduction

Previous studies have shown human breast cysts can be separated into two populations on the basis of the relative concentrations of Na^+ and K^+ in cyst fluid. One possible explanation is that the two cyst fluids may originate from different types of epithelium. The aim of this study was to investigate the relationship between the morphology of the epithelium lining human breast cysts and the content of cyst fluid.

Materials and Methods

Morphological assessment of the epithelium lining 40 human breast cysts was performed either by:

(i) centrifuging an aliquot of cyst fluid and examining the stained deposit cytologically (26 cysts from 22 patients), or

(ii) dissecting cysts from tissue obtained at mastectomy or biopsy, aspirating the cyst fluid and submitting the cyst wall for histological processing and serial sectioning (7 cysts from 6 mastectomy specimens and 7 cysts from 6 biopsy specimens).

The aspirates of cyst fluid were stained by Papanicolaou's technique (Pap) and by periodic acid Schiff after diastase digestion (PAS diastase). Dissected cyst specimens were stained by haematoxylin and eosin (H & E) and PAS diastase. PAS diastase positive granules in the cytoplasm of cells were used as a positive method of identification of apocrine epithelium (Azzopardi 1979). All cyst fluid aspirates and dissection specimens were assessed by a consultant pathologist with a specific training in breast histology and cytology.

The concentrations of Na^+ and K^+ in the 40 cyst fluids were measured by flame photometry and DHA sulphate was measured by radioimmunoassay.

Results

It was possible to identify two major types of epithelial cells in both cytological and dissection preparations.

(i) acidophilic cells (on H & E) with copious granular cytoplasm containing PAS diastase positive granules, luminal apical snouts (seen in histological preparations only) and nuclei showing prominent nucleoli - APOCRINE epithelium;

(ii) basophilic cells (on H & E) having less cytoplasm and containing no PAS diastase positive granules FLATTENED epithelium.

Figures 1 and 2 show examples of apocrine and flattened epithelium in breast cyst aspirates. Figure 3 shows the specific glycolipid granules in the cytoplasm of apocrine cells. Figures 4 and 5 demonstrate apocrine and flattened epithelium in dissection specimens and Figures 6 and 7 show PAS diastase stained sections of the two types of epithelium.

In this series, no cyst showed a mixture of apocrine and flattened epithelium in either aspiration or dissection specimens. In 22 cysts the lining was assessed as apocrine (14 by aspiration and 8 by dissection) and in 18 the lining was flattened epithelium (12 by aspiration and 6 by dissection).

The relationship between the morphology of the lining epithelium as assessed in cytological and dissection specimens and the ratio of Na^+ to K^+ is shown in Figure 8. Cysts lined by flattened epithelium all had a higher Na^+/K^+ ratio than cysts lined by apocrine epithelium, the difference in ratios being significant ($p < 0.001$ Wilcoxon's rank sum test). On the basis of the Na^+/K^+ ratio, it was thus possible to distinguish completely between apocrine and flattened epithelial cysts.

The DHA sulphate concentrations in cysts lined by the two types of epithelium are shown in Figure 9. The concentrations in the two groups was statistically significant with no overlap of values ($p < 0.001$ Wilcoxon's rank sum test).

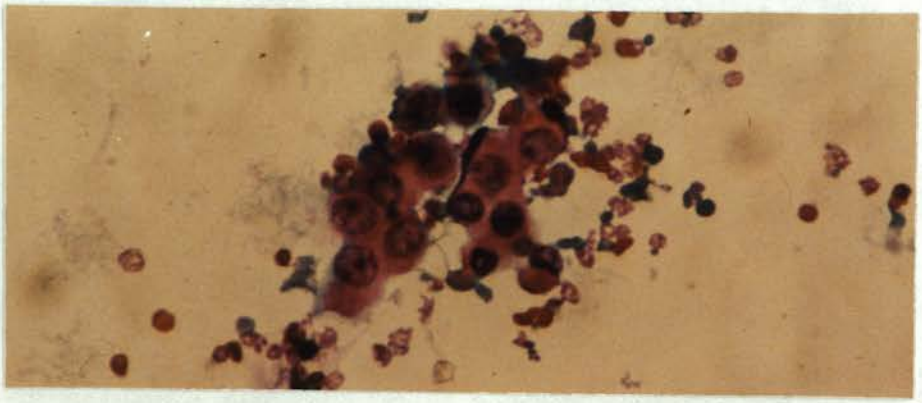


Figure 1 Cytological preparation of a cyst aspirate showing apocrine cells. The cells have copious pale cytoplasm with vesicular nuclei showing prominent nucleoli (Pap x 400)

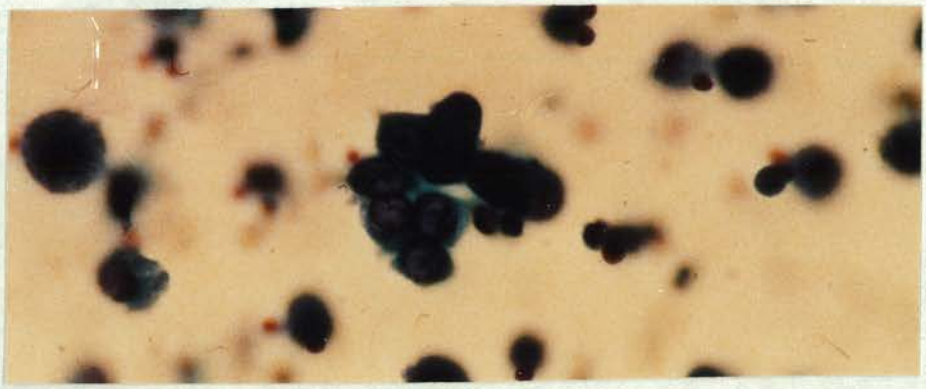


Figure 2 Cytological preparation of a cyst aspirate showing simple flattened epithelium. The cells have small amounts of cytoplasm, deeply staining nuclei and no special features (Pap x 400)

Figure 3 Cytological preparation of a cyst aspirate stained to show the specific glycolipid granules found in apocrine cells. The vesicular nuclei with prominent nucleoli also a feature of apocrine epithelium are easily seen (PAS diastase x 300)

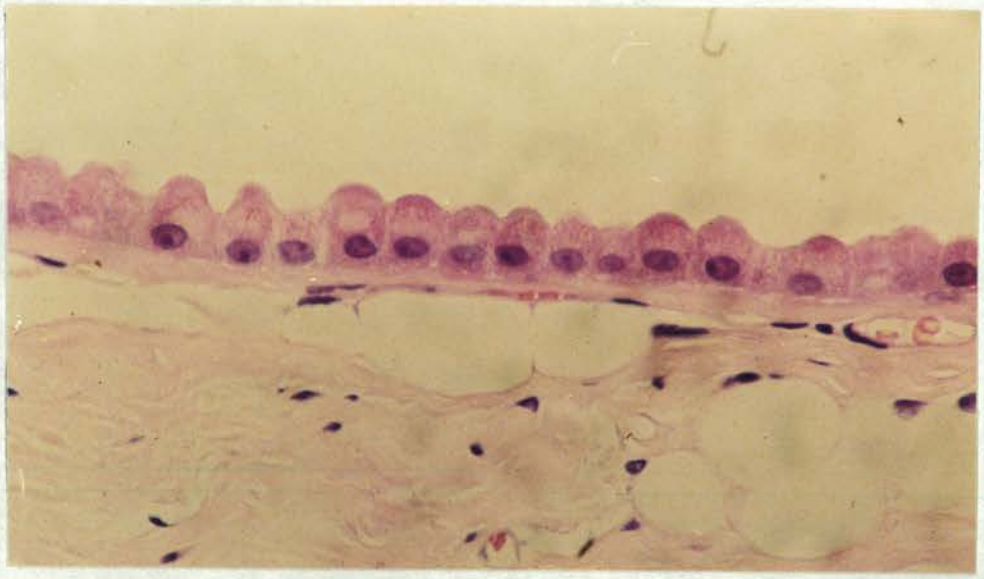


Figure 4 Dissection specimen of an apocrine cyst. The cells are columnar and have basally situated nuclei, abundant cytoplasm and apical snouts containing deeply staining intracytoplasmic glycolipid granules (H & E x 200)

Figure 5 Dissection specimen of a flattened epithelial cyst. The epithelium is attenuated and flattened and few cells are visible in any one section (H & E x 400)

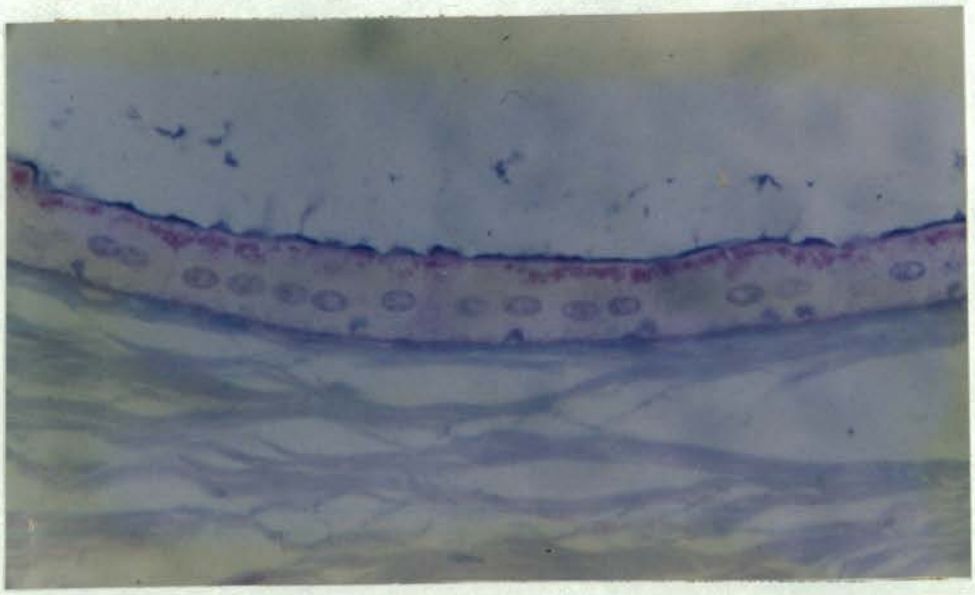


Figure 6 Dissection specimen showing the apical position of the glycolipid granules in apocrine epithelium (PAS diastase x 300)

Figure 7 Dissection specimen showing flattened epithelium stained to show glycolipid granules. Note the absence of any granules in this epithelium (PAS diastase x 400)

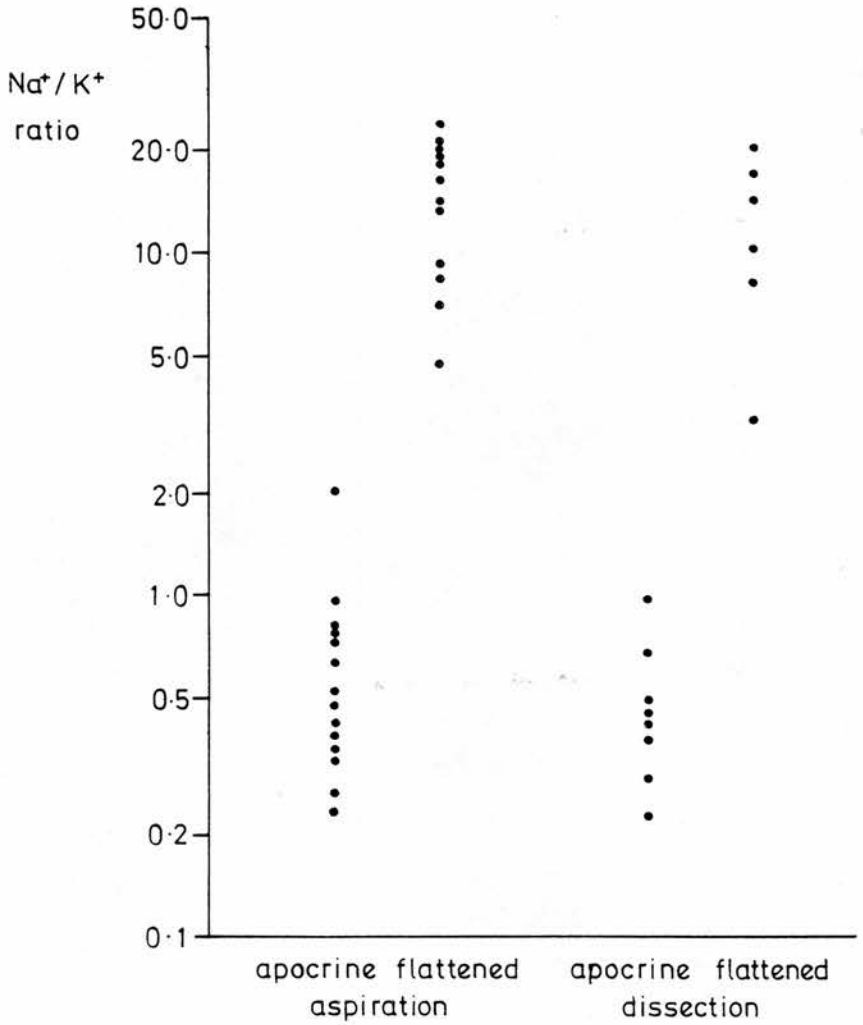


Figure 8 The ratios of Na+ to K+ in cysts where the epithelium was assessed as apocrine or flattened by aspiration and dissection techniques

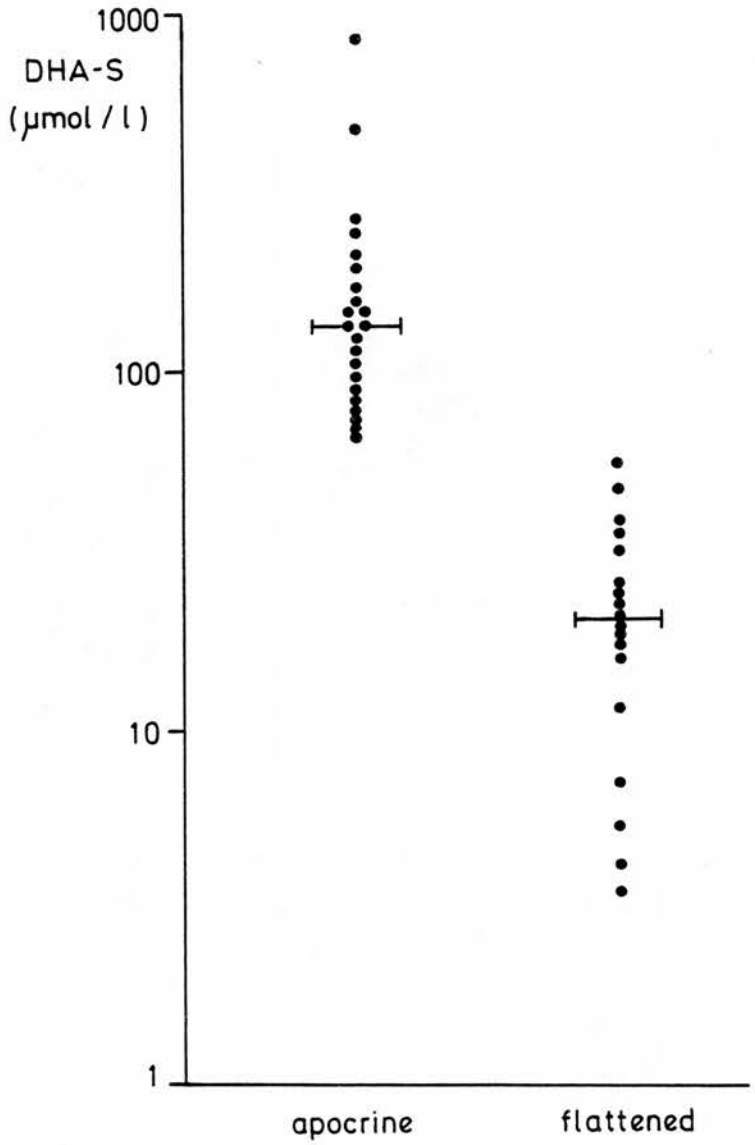


Figure 9 DHA sulphate concentrations in cyst fluids where the lining epithelium was assessed as apocrine or flattened.

(ii) Electron microscopy studies of breast cyst epithelium

The aim of this study was to compare and contrast the ultrastructural features of apocrine and flattened epithelium lining human breast cysts.

Patients, Materials and Methods

Biopsies from patients with cysts were processed as previously described and viewed by electron microscopy.

Results

Cysts lined by apocrine and flattened epithelium were identified. The main ultrastructural features of these two types of epithelium are outlined below.

Apocrine epithelium

The basally situated rounded nuclei with prominent nucleoli, and the apical snouts noted on light microscopy were more easily seen (Figure 10). The cytoplasm was rich in organelles with large numbers of mitochondria (Figure 11). The apical plasma membrane was lined by microvilli and below this, the apical cytoplasm contained membrane bound electron dense vesicles (Figure 12).

Flattened epithelium

The cells were elongated and attenuated and contained few organelles. There were few mitochondria and no membrane bound vesicles (Figure 13).

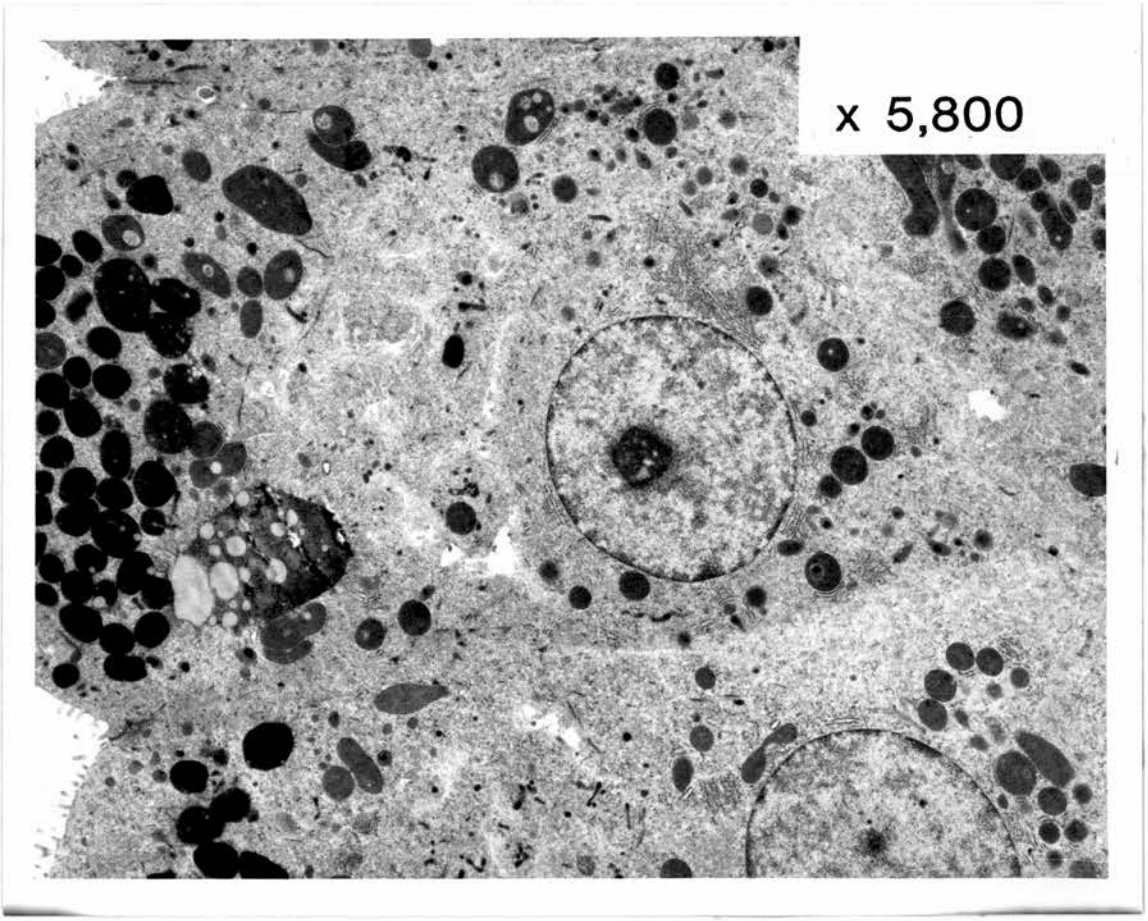


Figure 10 Electron microscopy of apocrine epithelium. The regular nuclei with prominent nucleoli and the cytoplasm rich in organelles are easily seen (x 5,800)

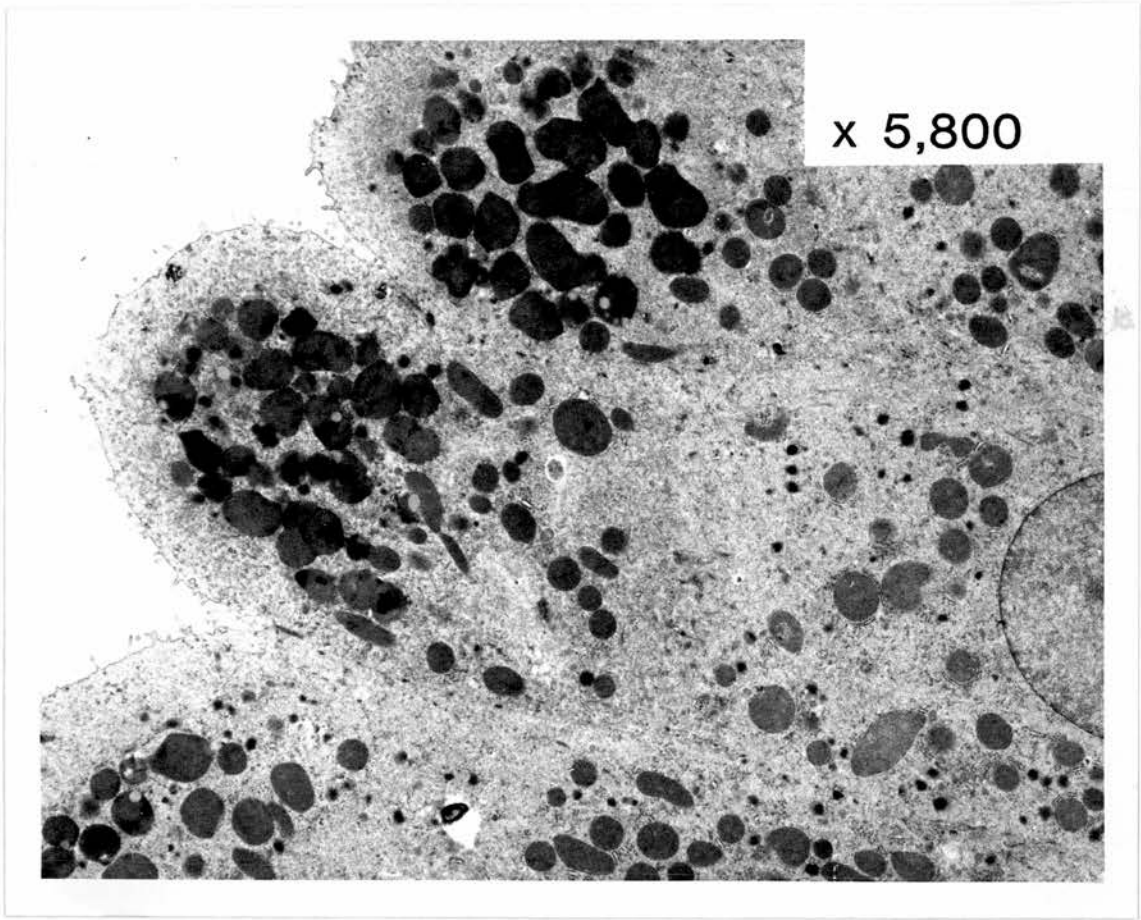


Figure 11 Electron microscopy of apocrine epithelium. Note the bulbous apical margins and the subapical electron dense intracytoplasmic granules (x 5,800)

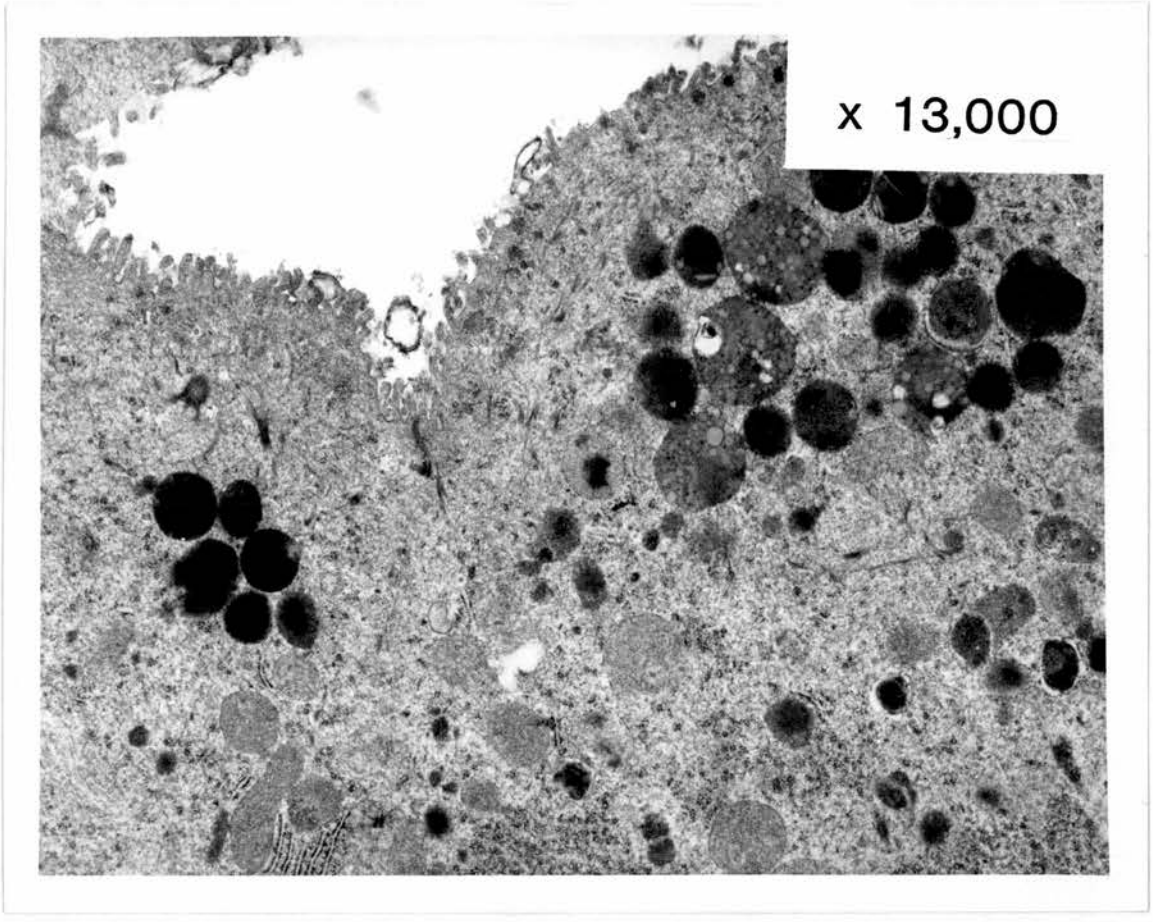


Figure 12 Electron microscopy of apocrine epithelium to demonstrate the microvilli and dense subapical granules (x 13,000)

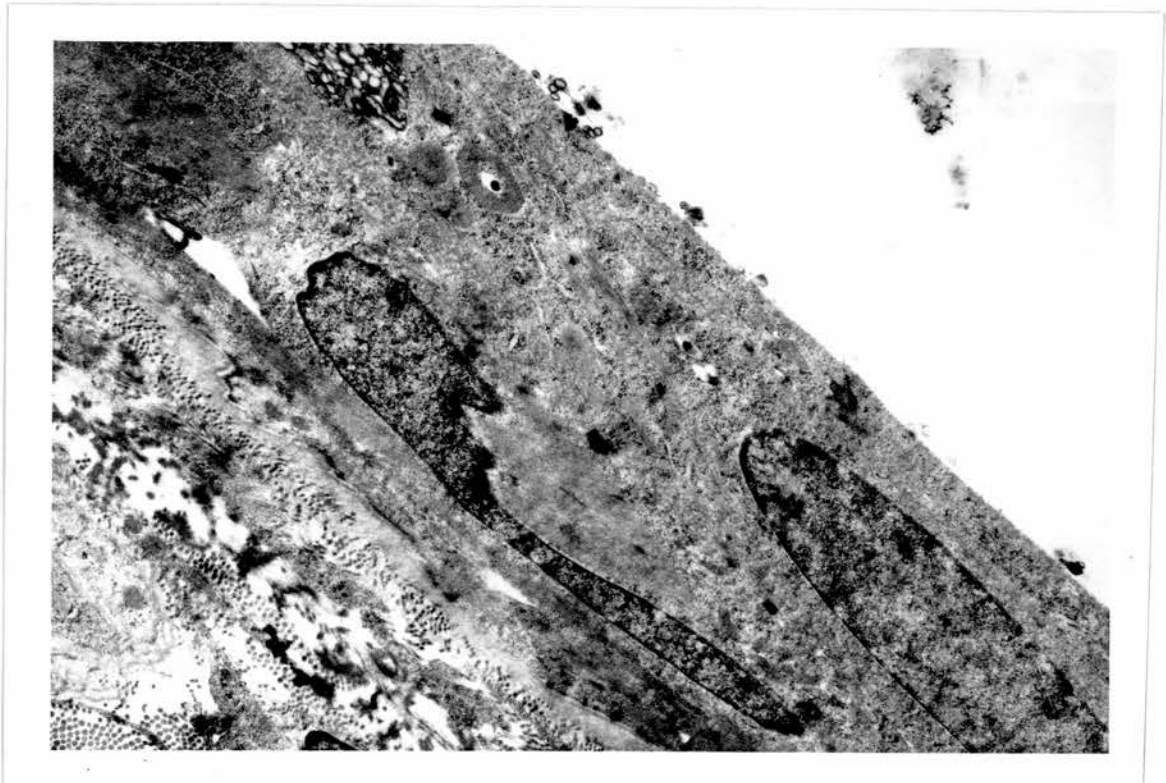


Figure 13 Electron microscopy of flattened epithelium. The cells are elongated, have no special features, no granules and no microvilli. A layer of myoepithelial cells is present beneath the flattened epithelium (x 20,000)

(iii) Peroxidase localisation of DHA sulphate in breast cyst epithelium

Introduction

Having identified that the two populations of cysts are lined by different types of epithelium and having previously shown differences in DHA sulphate concentrations in cyst fluids associated with these two types of epithelium, the aim of the present study was to determine if there were differences in the presence of this androgen conjugate in apocrine and flattened epithelium.

Materials and Methods

The material examined included:

(i) Frozen sections of biopsy and mastectomy material containing breast cysts (16 different portions of tissue from 10 different patients)

(ii) air dried cytological aspirates of breast cyst fluid (4 cyst fluids from 4 patients)

(iii) air dried cytological smears of apocrine epithelium (6 smears from 6 patients)

Figure 14 Preparation to demonstrate the presence of acid phosphatase a lysosomal enzyme in apocrine epithelium. The red colour represents the acid phosphatase activity which is greatest at the luminal aspect of the cell (x 200).

Results

Problems were encountered with the frozen section material. The epithelium lining the cyst was frequently difficult to section and either underwent lysis or floated off the slide during the performance of the peroxidase technique. This was particularly evident in apocrine epithelium which is known to contain high concentrations of lysosomal enzymes as demonstrated in Figure 14.

To obtain satisfactory results on the cytological deposits from cysts and from smears, the cells had to be frozen at -20°C before applying the primary antibody.

It was, however, evident that apocrine epithelium in smears or aspirates from cysts contained large amounts of material cross-reacting with the antibody (Figures 15-17). This was diffusely distributed in the cytoplasm, but occasionally was more prominent in the supranuclear portions of the cells and appeared granular. In contrast, normal epithelial cells (Figure 18), other tissues in the sections and the cells lining flattened cysts (Figures 19 and 20) showed little or no evidence of staining by this technique.

Figure 15 Frozen section of apocrine epithelium stained to show the presence of DHA sulphate by the immunoperoxidase stain (x 200)

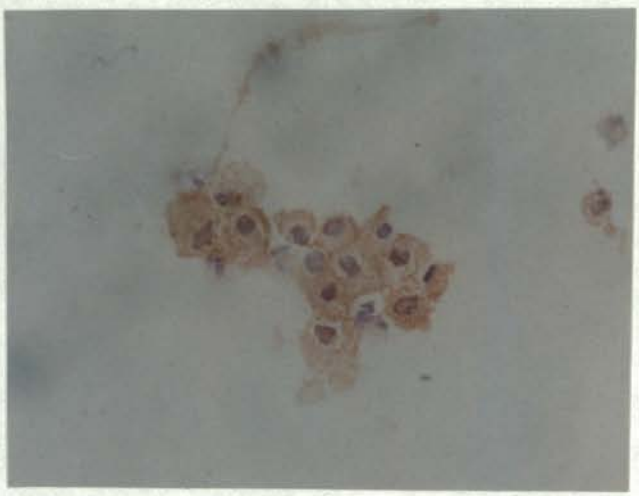


Figure 16 Apocrine epithelium from a breast cyst stained for DHA sulphate (cytocentrifuge preparation x 400)

Figure 17 Apocrine epithelium in a fine needle aspirate specimen stained for DHA sulphate (x 400)

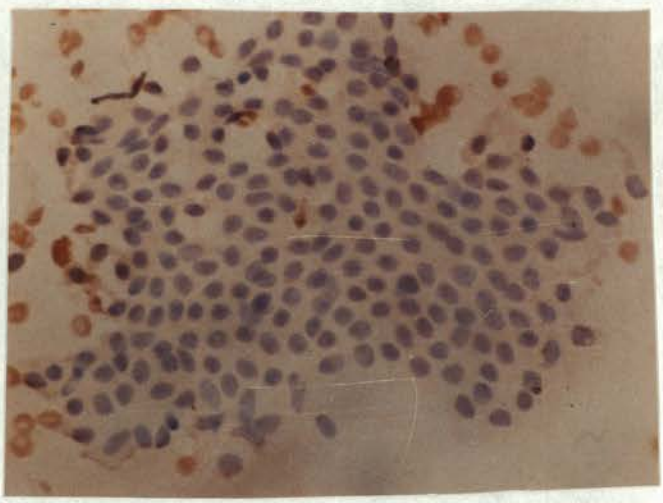


Figure 18 Normal epithelial cells in a smear stained for DHA sulphate. Note the lack of staining (x 400)

Figure 19 Flattened epithelium stained for DHA sulphate, little or no staining is evident (frozen section x 200)

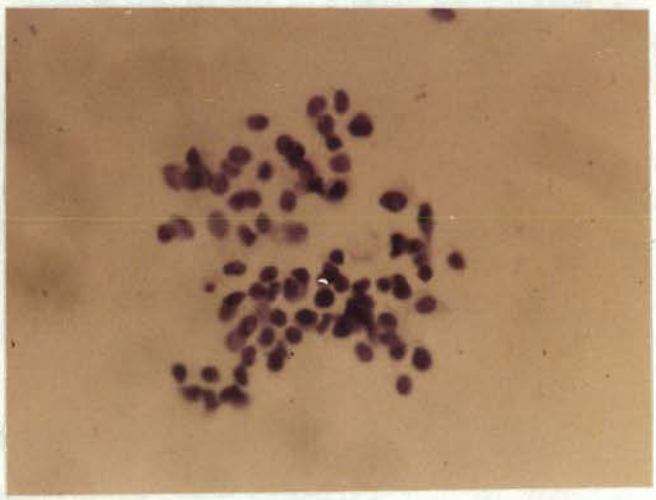


Figure 20 Flattened epithelial cells in a cyst aspirate stained for DHA sulphate (cytocentrifuge preparation x 400)

DISCUSSION

The two major populations of cysts which can be defined on the basis of the relative concentrations of Na^+ and K^+ are lined by different types of epithelium. One group of cysts is lined by apocrine epithelium and cyst fluid derived from this epithelium has been shown to contain high concentrations of K^+ and DHA sulphate. It is of note that apocrine secretions from axillary skin have high concentrations of DHA sulphate (Labows et al 1979). Apocrine sweat is also known to vary in colour from yellow to green to blue-black (Hurley et al 1960), a similar colour range to that seen in cyst fluids. The other group of cysts is lined by flattened epithelium and, as previously noted, has a composition which more closely resembles that of plasma.

Apocrine epithelium has been shown to have a high content of oxidative enzymes (Ahmed et al 1975). The large numbers of mitochondria present in the cytoplasm of apocrine cells is consistent with this. The presence of vacuoles beneath the luminal surface of these cells suggests that the material in these may be expelled into the lumen. This type of activity indicates merocrine rather than apocrine secretion, the latter occurring by budding off apical cytoplasm. These observations and interpretations have also been made by others (Pier et al 1970, Ahmed 1975, Ahmed 1979, Azzopardi 1979). It has also been noted in ultrastructural studies that there are prominent blood vessels adjacent to apocrine epithelium (Ahmed et al 1975). All these observations suggest that

apocrine epithelium is very active and secretory and correlates well with the findings of high concentrations of secretory products in cyst fluid derived from this epithelium. These findings are inconsistent with the view of Dawson (1932) that apocrine cells arise by the result of degeneration and those of Bonser et al (1961) who consider that apocrine epithelium occurs in areas with a poor blood supply. In marked contrast to apocrine epithelium, ultrastructural studies of flattened epithelium showed few organelles and no introcytoplasmic secretory granules.

This study has also demonstrated that high concentrations of androgen conjugates are present within the cytoplasm of apocrine epithelium and this correlates well with the findings of high levels in cyst fluid derived from this epithelium. The distribution of DHA sulphate is rather granular in the cytoplasm of these cells and appears in a similar distribution to the glycolipid granules detected by the PAS diastase technique. It has been shown that labelled DHA sulphate in blood is concentrated in cyst fluid and it may be that this is transported across the cell in these granules (Bradlow et al 1983a). Such active concentration would require large amounts of energy which the mitochondria in the cell could furnish.

The presence of DHA sulphate in apocrine cyst fluid, axillary apocrine sweat glands and the cytoplasm of apocrine epithelium in the breast indicates that this compound may be a marker of apocrine activity. Petrakis (1977) has suggested a link between apocrine

activity and breast cancer; the higher the activity, the greater the incidence of breast cancer. Other authors have also shown that apocrine change in the breast occurs more frequently in patients at risk of breast cancer (Wellings et al 1975, Haagensen et al 1979, Schuerch et al 1982, Roberts et al 1984). The measurement of DHA sulphate or of electrolytes in cyst fluid which both indicate the presence of apocrine activity in the lining epithelium, may therefore be helpful in identifying patients at increased risk of breast cancer.

Relationship of composition of breast cyst fluid and cyst epithelial lining to behaviour

(i) in relation to the natural history of cystic disease

(ii) in relation to hyperplasia

(iii) in relation to breast carcinoma

(i) Relationship of composition of breast cyst fluid and epithelial lining to the natural history of cystic disease

Introduction

Some 7% of all women develop a palpable breast cyst (Haagensen et al 1981). It is known that about half of all women who present with cystic disease have a single cyst, a third of patients develop between 2 and 5 cysts and the remainder have in excess of 5 (Haagensen et al 1981). No factor is known which determines whether a patient will develop a single or multiple cysts. The aim of the present study was to determine if there are differences in natural history of the two cyst types defined on electrolyte composition of cyst fluid and cyst epithelial lining.

Patients

A prospective analysis has been carried out of 100 consecutive patients presenting for the first time with a breast cyst and followed over at least a 2 year period. All cysts aspirated from these 100 patients were classified on the basis of the Na^+/K^+ ratio in cyst fluid as apocrine if $Na^+/K^+ < 3$ or flattened if $Na^+/K^+ \geq 3$. Statistical comparison of groups was by the X^2 test.

RESULTS

Of the 100 patients, 45 had only 1 cyst, 46 developed between 2 and 5 cysts and 9 had in excess of 5. There were a total of 247 cysts aspirated during the period of follow-up, 177 of which were classified as apocrine and 70 as flattened. Forty-three patients developed single or multiple cysts of flattened type, 44 patients developed single or multiple cysts of apocrine type and 13 patients had mixtures of the two types of cysts.

Table I shows the percentage of cysts which were classified as apocrine and flattened in patients with 1, 2-5 and > 5 cysts. It can be seen that patients with a single cyst were more likely to have a flattened cyst and, as the number of cysts aspirated per patient increased, so the proportion of apocrine cysts also increased. This is more clearly illustrated when the ratio of apocrine:flattened cysts is plotted for the groups with varying numbers of cysts as in Figure 1.

There were 56 episodes where patients had multiple simultaneous cysts and in 48 (88%), all cysts were of the same type, ie all apocrine or all flattened. Forty-three patients developed sequential cysts and in 32 (74%) of these, all cysts aspirated over the two year period were of the same type. Thus, individual patients who have multiple cysts, whether they are simultaneous or sequential usually develop cysts of one or other type. From the

Number of cysts aspirated	Number of patients	% of cysts which were	
		Apocrine	Flattened
1	45	24	76
2-5	46	75	25
> 5	9	94	6

Table I Comparison of the % of cysts which were classified as apocrine or flattened on the basis of electrolyte composition in groups of patients who had totals of 1, 2-5 or > 5 cysts aspirated over a 2 year period.

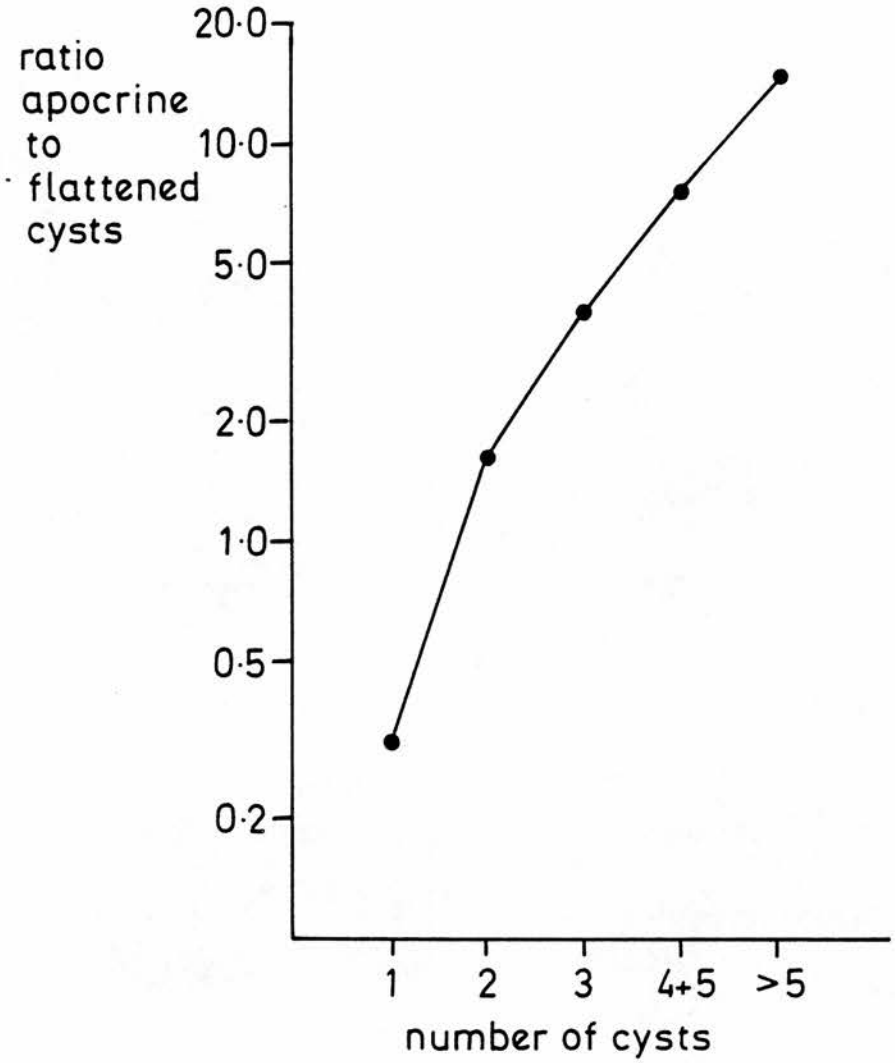


Figure 1 Ratio of apocrine: flattened cysts in the groups of patients who had 1, 2, 3, 4 & 5 and >5 cysts aspirated over the follow-up period

data presented above, it is also evident that this type is much more likely to be apocrine.

Sixty-nine patients originally presented with a single cyst, 30 of which were classified as apocrine and 39 as flattened; 24 patients developed further cysts, 19 of the 30 (63%) with apocrine and 5 of the 39 (13%) with flattened cysts. The difference between further cysts in the two groups was highly significant ($p < 0.0005$). Overall 14% of those presenting initially with flattened cysts, 72% of those presenting with apocrine cysts and 50% of those with mixtures of apocrine and flattened cysts went on to develop further cysts (Table II). Thus, patients presenting with apocrine cysts were more than 5 times more likely to develop a further cyst than those with flattened cysts. Of the patients who developed further cysts, 85% of the patients who initially had apocrine and 50% of the patients who originally had flattened cysts developed subsequent apocrine cysts.

There therefore appears to be great differences in the subsequent behaviour of apocrine and flattened cystic disease.

Cyst type at presentation	Number of patients	Number developing further cysts	% developing further cysts
All flattened	47	6	14%
All apocrine	47	34	72%
Mixtures of 2 types	6	3	50%

Table II Comparison of numbers of patients who developed further cysts during a two year follow-up in the groups who initially presented with either all flattened, all apocrine or mixtures of the two types of cysts.

(ii) Relationship of epithelial lining of breast cysts to hyperplasia

Introduction

Epithelial hyperplasia is a component part of cystic disease and has been shown to be one of the main factors associated with the increased risk of breast cancer in this group of patients (Kern et al 1969, Steinhoff et al 1970, Black et al 1972, Kodlin et al 1977, Page et al 1978, Azzopardi 1979, Roberts et al 1984). This finding comes from pathological studies of biopsies performed in patients with cystic disease, but the majority of women with this condition are never biopsied and are treated by simple aspiration (Haagensen et al 1981). It remains uncertain which of these women are at risk of breast cancer. The aim of the present study was to compare the frequency and degree of hyperplasia in the groups of patients with apocrine and flattened cysts.

Patients, Materials and Methods

During the early 1960's, it was practice in Edinburgh to biopsy all breast lumps. From the years 1966 and 1967 all patients who were considered clinically to have a palpable cyst and who underwent biopsy were identified and the histology of all of these biopsies reviewed. The lining epithelium of any macrocysts (> 3 mm) was recorded, as were any pathological changes in the surrounding breast tissue. Particular attention was paid to the presence and numbers

of any microcysts, the presence of foci of apocrine change and papillary apocrine change, adenosis and any hyperplasia. Hyperplasia, when present, was assessed on a 5 point scale using similar criteria to that of Wellings et al (1975). Grade I normal, II minimal or moderate hyperplasia, III severe hyperplasia, IV hyperplasia with atypia and V carcinoma in situ.

Statistical analysis comparing the frequency of pathological features in the two groups was by either the Wilcoxon rank sum or the X^2 test.

Results

One hundred and sixteen patients underwent biopsy for a single or multiple breast cysts during 1966 and 1967 in the Royal Infirmary of Edinburgh. Eighty of these had a total of 183 apocrine cysts, 30 had 38 flattened cysts and 6 patients had mixtures of the two types of cysts. There were thus an average 2.3 macrocysts per patient in the group with apocrine cysts, significantly more than the 1.3 macrocysts per patient in the group with flattened cysts, $p < 0.0005$.

A summary of the pathological changes in the biopsy specimens from the 110 patients who had all macrocysts lined by either only apocrine or only flattened epithelium is presented in Table III. Microcysts, papillary apocrine change and hyperplasia were all significantly more common in the group with apocrine cysts.

CYST EPITHELIUM	NUMBER OF PATIENTS	NUMBER AND PERCENTAGE OF PATIENTS IN EACH GROUP WITH				
		MICROCYSTS	OTHER FOCI OF APOCRINE CHANGE	PAPILLARY APOCRINE CHANGE	HYPERPLASIA GRADE II	HYPERPLASIA GRADE III + IV
Apocrine	80	49* (61%)	68* (85%)	49* (62%)	41* (51%)	9† (11%)
Flattened	30	10 (33%)	12 (40%)	8 (27%)	8 (27%)	0 (0%)

Table III Comparison of the frequency of a variety of pathological changes in the two groups of patients who, on biopsy, were shown to have macrocysts all lined by either apocrine and flattened epithelium.

*significantly greater frequency in apocrine group * $p < 0.0005$. † $p < 0.05$.

Hyperplasia of a severe degree (Grade III) or with atypia (Grade IV) were only seen in patients with apocrine cysts.

Thus, patients with apocrine cysts are more likely to have in their breasts a variety of other pathological changes which includes both papillary apocrine change and epithelial hyperplasia.

(iii) Relationship of breast cyst fluid composition and cyst epithelial lining to breast cancer

Introduction

It is well recognised that breast cancer may develop in patients with cystic disease (Haagensen et al 1981). No study has, however, studied the composition of cysts or the nature of the epithelium in cyst fluid aspirated from women who have later developed breast cancer. It has been practice in this department for a number of years either to send cyst fluid for cytology or to store cyst fluid for later analysis. The aim of the present study was to classify those cysts aspirated from patients who, at a later date, developed breast cancer.

Patients, Materials and Methods

From a review of 400 patients who developed breast cancer, 11 patients were identified who had a history of cyst aspiration and where all cysts aspirated could be classified either on the basis of cytology (5 patients) or electrolyte composition (6 patients) as apocrine or flattened. The ratio of apocrine : flattened cysts in this group of women has been compared with that from the series of 100 patients followed over 2 years using the X^2 test.

Results

The 11 patients had 24 cysts aspirated. Three of these had only a single cyst, 5 had 2 cysts aspirated, 1 had 3 and 2 developed 4 cysts. Ten patients had single or multiple apocrine cysts and 1 patient had a single flattened cyst. This ratio of 10:1 apocrine:flattened compares with the ratio of 44:43 in the series of 100 patients presented earlier. These ratios are significantly different ($p < 0.02$) and this indicates that patients with breast cancer are more likely to have had apocrine rather than flattened cysts and suggests that breast cancer may develop more commonly in patients with apocrine cysts.

DISCUSSION

Two populations of human breast cysts have been identified. These are lined by either apocrine or flattened epithelium and differ in electrolyte composition of contained cyst fluid. The present study has shown that these two types of cysts are associated with differences in natural history, association with risk factors for breast cancer and the development of breast cancer.

It has been shown that cysts lined by apocrine epithelium contain high concentrations of secretory products indicating an active epithelium. Patients with these active cysts are more than 5 times more likely to develop further cysts than those with cysts lined with flattened (inactive) epithelium. It therefore seems likely that activity of lining may be an important factor in cyst formation. It is of interest that, although multiple cysts are much more likely to be apocrine, multiple flattened cysts do occur and only a minority actually develop mixtures of the two types of cysts. Thus, there appears to be a factor in each patient which determines whether she will develop cysts lined by flattened or apocrine epithelium.

Patients with greater than 5 cysts have almost exclusively apocrine cystic disease. A number of treatments have been tried to reduce the frequency of cysts in these patients without great success. From this study, it is apparent that an agent which reduces apocrine secretory activity may reduce the frequency of cyst formation. The

oral contraceptive has been reported to reduce apocrine secretion (Royal College of General Practitioners 1974). Earlier studies reported in this thesis showed that patients taking the oral contraceptive at the time of developing a cyst were (i) less likely to have an apocrine cyst, and (ii) significantly less likely to develop further cysts. Other drugs capable of reducing apocrine secretion may therefore prove of use in the treatment of recurrent breast cysts.

It has been suggested that women with cystic disease are at increased risk of subsequent breast cancer (Azzopardi 1979, Haagensen et al 1981). The factors which have been associated with this increased risk include hyperplasia, epithelial atypia and papillary apocrine change (Kern et al 1969, Steinhoff et al 1970, Black et al 1972, Monson et al 1976, Kodlin et al 1977, Page et al 1978, Azzopardi 1979, Haagensen et al 1981, Roberts et al 1984). This study has clearly shown that all of these changes are significantly more common in patients with apocrine cysts when compared to those with cysts lined by flattened epithelium. Other workers have also noted this association of apocrine epithelium and hyperplasia (Foote et al 1945, Wellings et al 1975, Haagensen et al 1981, Vilanova et al 1983).

In keeping with this association of risk factors for breast cancer and apocrine cysts, this study has also shown that patients with breast cancer are more likely to have had apocrine rather than flattened cysts aspirated prior to presenting with cancer. It is of

interest in this regard that, in populations which have a high frequency of breast cancer, apocrine change within the breast is more common than in populations with a low risk (Schuerch et al 1982). As the incidence of apocrine change varies in different parts of the world, the proportion of apocrine and flattened cysts may also vary and this may account for some of the geographical variation of incidence of breast carcinoma. It has also been reported that women with gross cysts who have histological evidence of apocrine change are more than 11 times more likely to develop breast cancer than those women with cysts with no histological evidence of apocrine change (Haagensen et al 1981). It is also relevant that it is those women who have more than one cyst aspirated who may be at increased risk of breast cancer (Haagensen et al 1981). This study shows that these women are likely to have apocrine cystic disease.

It is important to point out that the findings of the present study in relation to breast cancer can only be compared with those of Haagensen (1971, 1981). This is the only other data relating breast cancer to cystic disease treated by aspiration. All other reports relate to the histological studies of biopsy material but, as the majority of patients with cystic disease are not biopsied, the findings of these studies are therefore not directly comparable.
The natural history of breast cystic disease and risk of subsequent breast cancer appears directly related to whether the patient develops cysts lined by apocrine or flattened epithelium. The majority of women with flattened cysts have only a single cyst and

appear at little risk of breast cancer, whereas those women with apocrine cysts tend to have multiple cysts and appear at a relatively increased risk of breast cancer.

Origin of Cysts

(i) Studies on the composition of human breast microcysts

(ii) Histological and ultrastructural studies of microcyst epithelium

(iii) Peroxidase localisation of DHA sulphate in microcyst epithelium

Introduction

Clinically palpable breast cysts appear to be derived by microcysts (< 0.3cm) (Haagensen et al 1981, Schwartz 1983) which arise from breast lobules (Wellings et al 1975, Wellings 1980). Two populations of human breast macrocysts have been identified and these differ in electrolyte and hormonal composition. Little, however, is known of the content of human breast microcysts and, in particular, it is not known if there exists a single or dual populations. The aim of this study was to analyse the content of human breast microcysts.

Patients, Materials and Methods

Forty breast microcysts were dissected from 8 biopsy and 14 mastectomy specimens prior to fixation using a dissecting microscope. Figure 1 shows a microcyst in a gross biopsy specimen. Figures 2 and 3 show a microcyst in situ and after dissection as seen through the dissecting microscope. The fluid within the microcysts were collected after puncture into calibrated capillary tubes (Figure 4). The volume of microcyst fluid was calculated (range 0.5 - 20 μl) by measuring the length of sample in the capillary tube. The fluid was then diluted to 100 μl in distilled water and stored at -40^{o}C for later analysis.

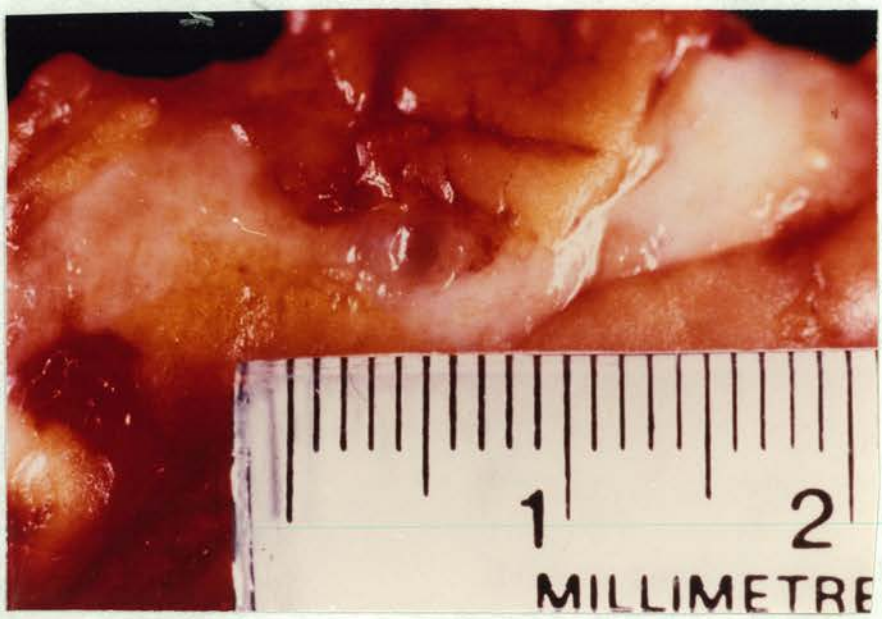


Figure 1 A microcyst in a gross biopsy specimen

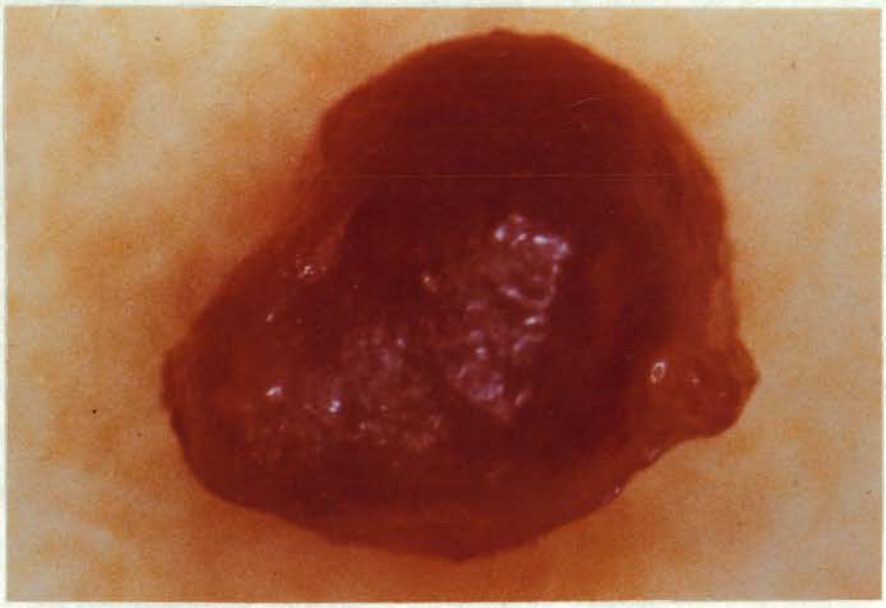


Figure 2 A microcyst dissected from a biopsy specimen (x 32)

Figure 3 A microcyst after removal of fluid (x 20)

Figure 4 Microcyst fluid collected in a capillary tube (x 20)

DHA sulphate was estimated in all 40 microcysts by radioimmunoassay. Na+ and K+ concentrations were measured in the 10 cyst fluids where sufficient fluid was available (> 10 μl) by flame photometry.

Results

The concentration of DHA sulphate in fluids from the 40 microcysts is shown in Figure 5. For comparison, data are included on the ranges and median values of DHA sulphate in plasma and fluid from macrocysts lined by apocrine and flattened epithelium. All microcyst fluids contained levels of DHA sulphate at least 10 times greater than the upper limit of normal for plasma and the median value was almost 1000 times greater than that in plasma. Fluids from more than half the microcysts contained concentrations of DHA sulphate greater than that seen in any macrocysts. All microcysts also contained DHA sulphate concentrations higher than the range of fluids from cysts lined by flattened epithelium.

All 10 microcysts contained concentrations of K+ in excess of those of Na+. The Na+/K+ ratio of the 10 microcysts in which electrolyte composition was estimated is compared with that of the ratio in apocrine and flattened epithelial macrocysts in Figure 6. All 10 microcysts had a Na+/K+ ratio in the range of that seen in apocrine cysts.

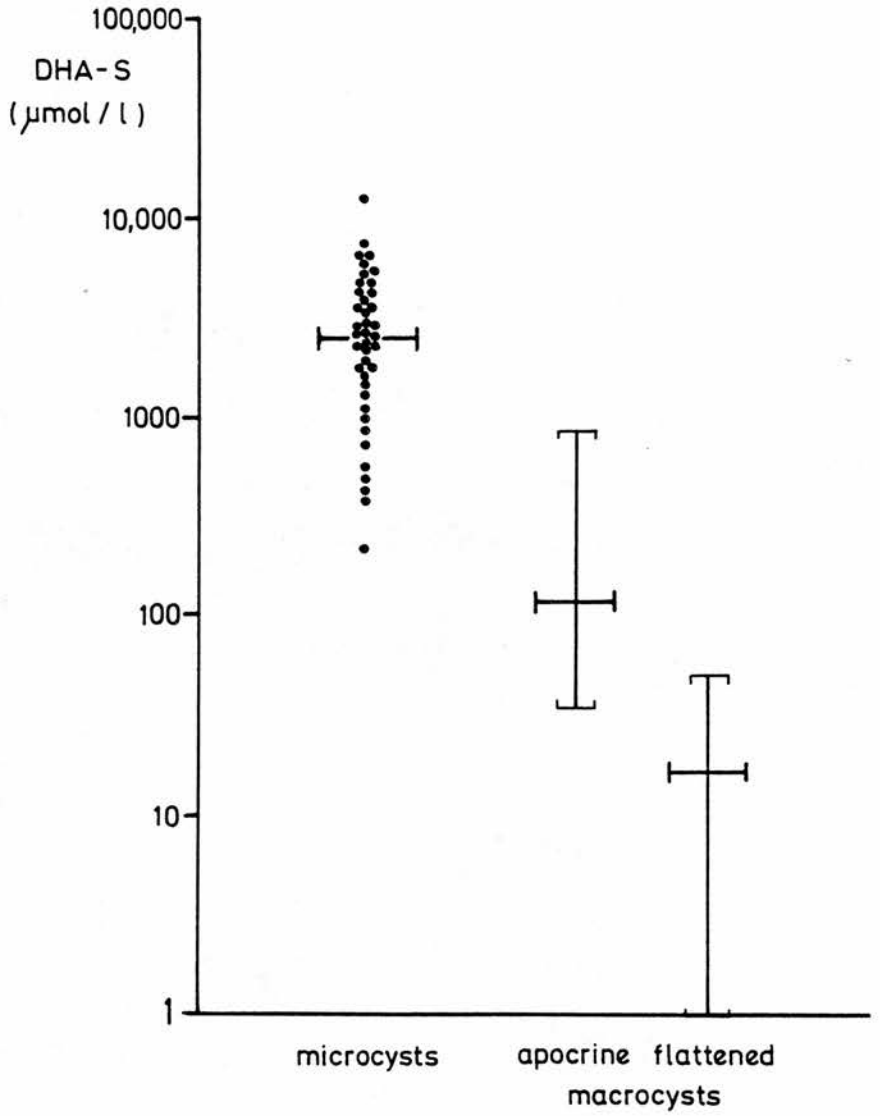


Figure 5 Concentration of DHA sulphate in breast microcyst fluid. Horizontal bar represents median value. The ranges and median values for the DHA sulphate concentrations of apocrine and flattened macrocysts and plasma are included for comparison

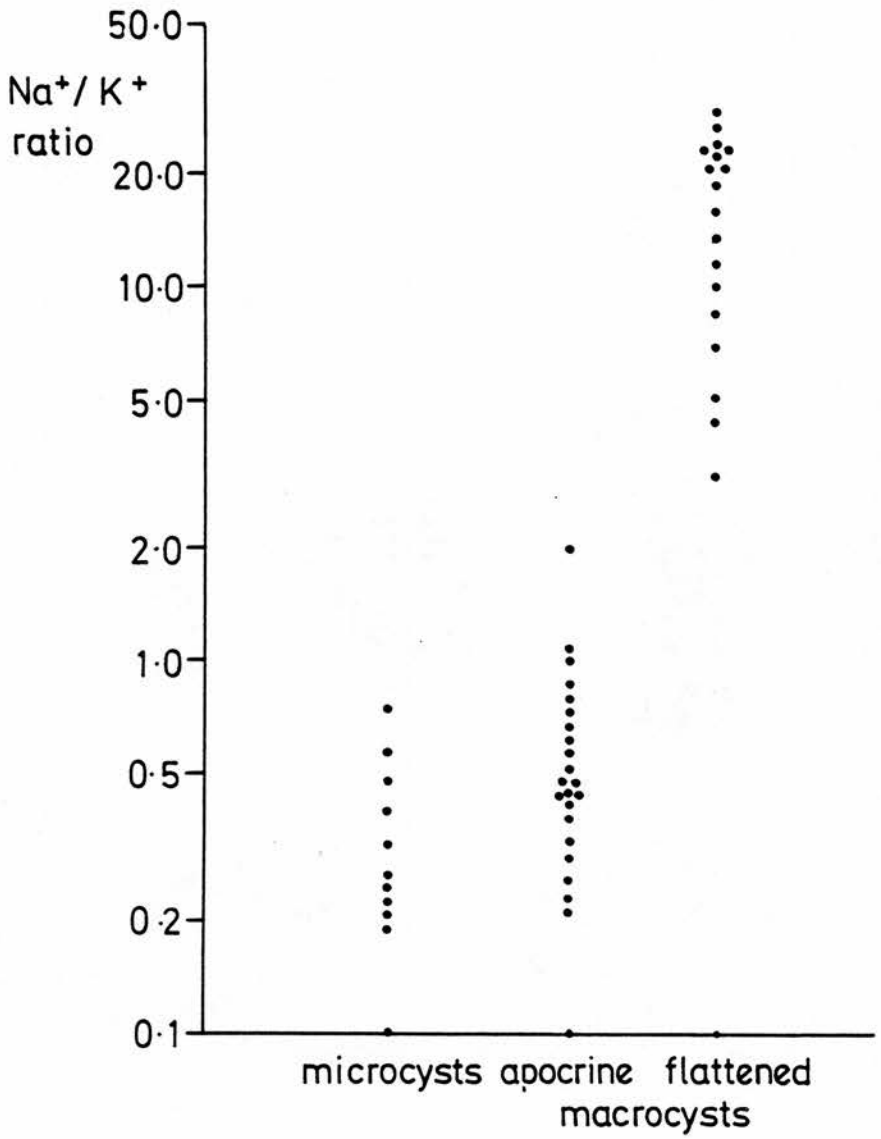


Figure 6 The Na+/K+ ratio in microcyst fluid. The range of ratios for apocrine and flattened macrocysts are included for comparison

(ii) Histological and ultrastructural studies of microcyst epithelium

The aim of this study was to determine the nature of the epithelium lining breast microcysts.

Materials and Methods

The 40 microcysts from which fluid was removed were fixed in 10% formalin solution, histologically processed and serial sections cut and stained by H and E and PAS diastase techniques. PAS diastase positive granules within the epithelium lining the microcysts was used as a positive method of identifying apocrine epithelium (Azzopardi 1979).

Microcysts in 2 biopsy and 2 mastectomy specimens were examined by electron microscopy. A total of 20 microcysts were studied.

Results

All 40 microcysts were lined by epithelium which showed apocrine features. Many showed columnar epithelium with apical snouts (Figure 7) but, in others, the epithelium was more cuboidal. All contained, to some degree, PAS diastase positive granules at the luminal margins of the lining epithelium (Figure 8).

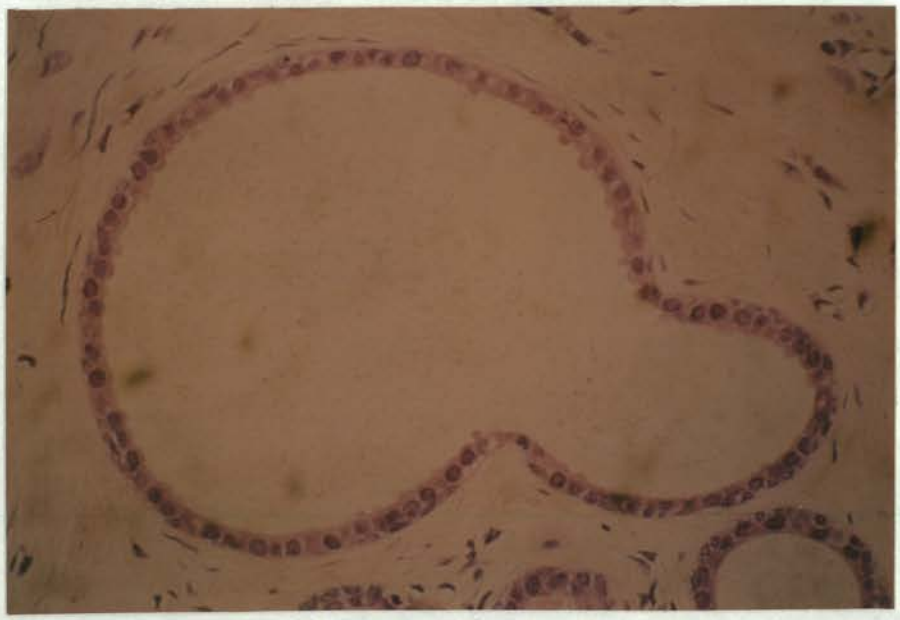


Figure 7 A breast microcyst. The columnar epithelium showing many apocrine features (basal nuclei, apical snouts) is seen (H & E x 40)

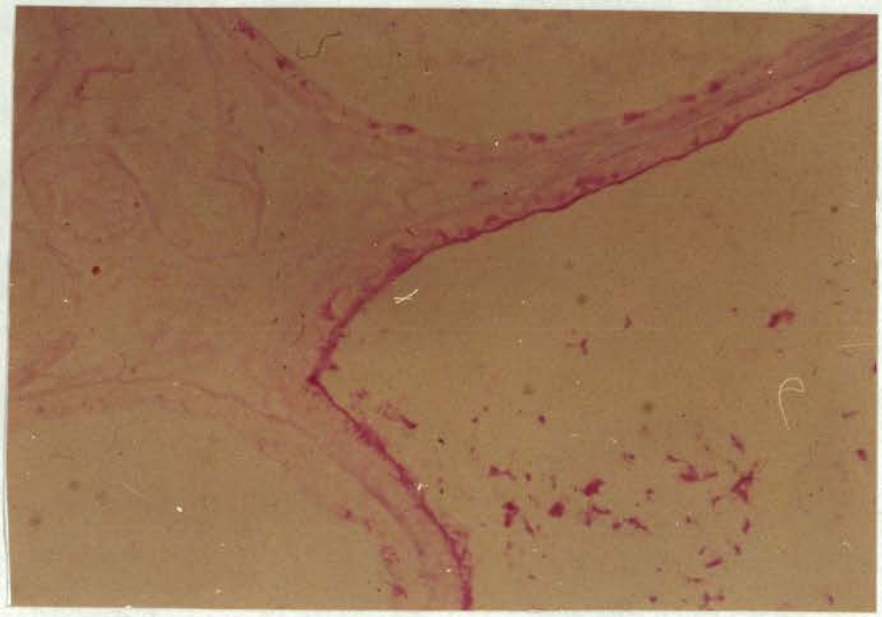


Figure 8 Microcyst epithelium stained to show the glycolipid granules present in the epithelium (PAS Diastase x 400)

Ultrastructural studies confirmed the apocrine nature of the epithelium of microcysts even when cuboidal (Figure 9). In some microcysts, part of the epithelium was tall and apical snouts were present, while in other areas, the cells were more elongated. However, even in the elongated epithelium, dense granules, numerous mitochondria and apical microvilli were still present and thus these cells differ from those lining flattened macrocysts which have none of these features.

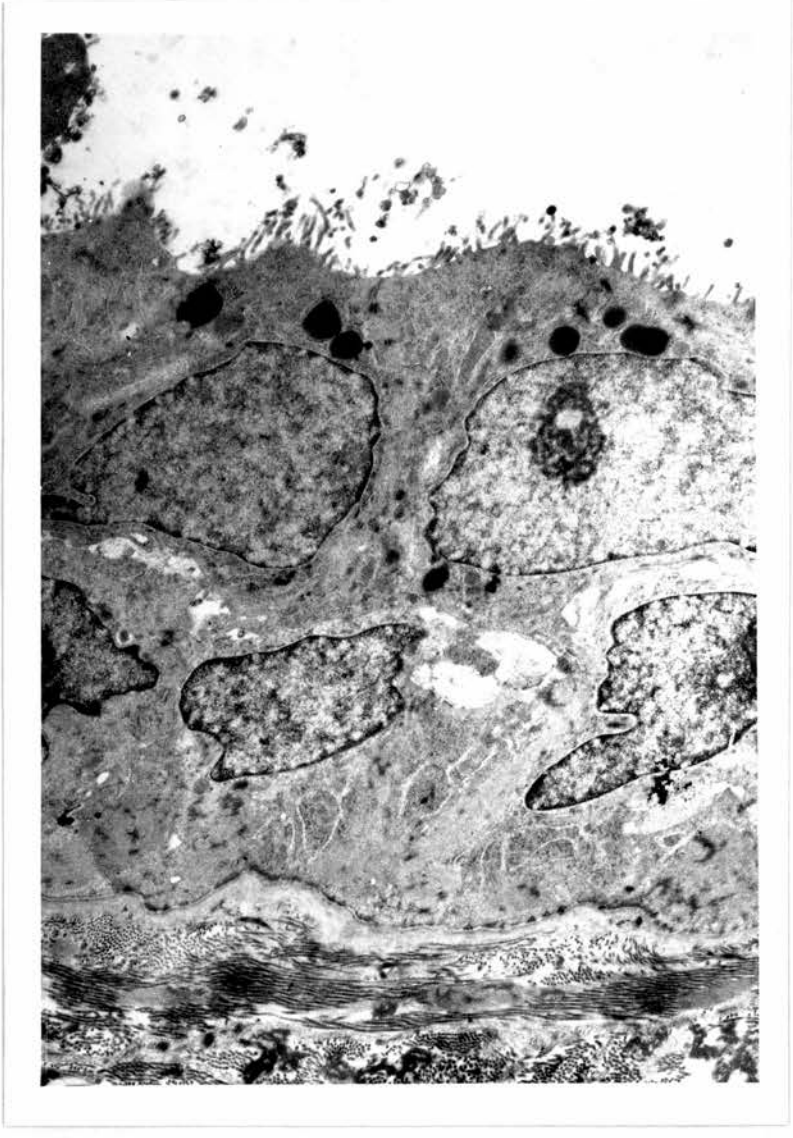


Figure 9 Electron microscopy of microcyst epithelium. The microvilli the electron dense intracytoplasmic granules and the nucleus with a prominent nucleolus all features of apocrine epithelium are present in typical microcyst epithelium (x 8,000)

(iii) Peroxidase localisation of DHA sulphate in microcyst epithelium

Having identified high concentrations of DHA sulphate in the fluid derived from microcyst epithelium, the aim of the present study was to determine if high concentrations of DHA sulphate are present within the cytoplasm of these cells.

Materials and Methods

Frozen sections from six biopsy and two mastectomy specimens containing 14 microcysts were obtained. DHA sulphate was localised in these sections by the immunoperoxidase technique previously described.

Results

All 14 microcysts in the eight specimens contained material in the cytoplasm of the cells which stained positively with the anti-DHA sulphate antibody. An example of the deep staining seen in some microcysts is shown in Figure 10. In these sections the normal epithelium failed to stain.

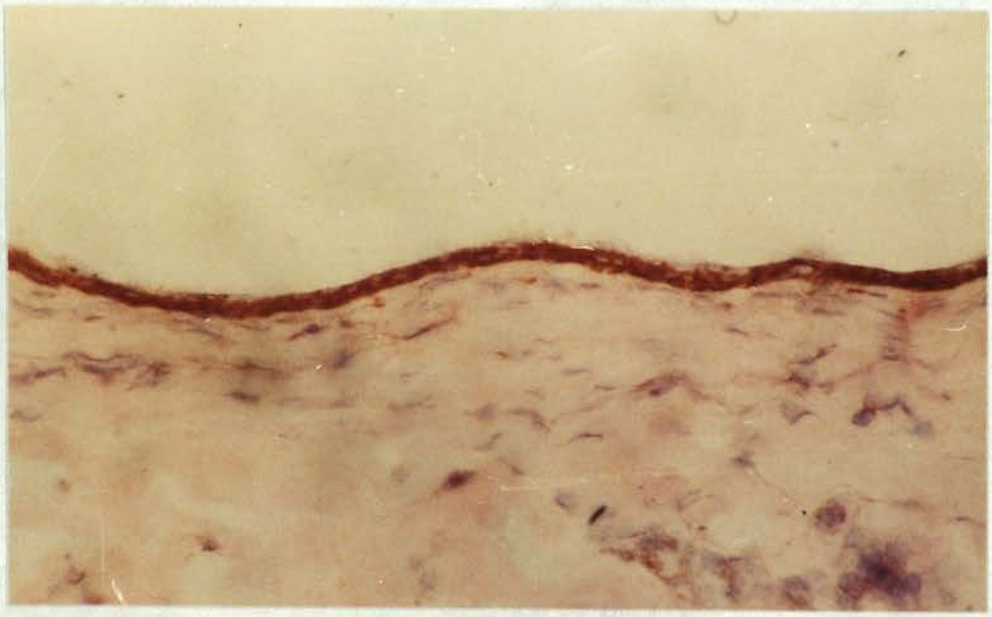


Figure 10 Microcyst epithelium stained for DHA sulphate by the immunoperoxidase technique. The prominent staining indicating a high concentration of DHA sulphate is easily seen (frozen section x 400)

Discussion

The present study has shown that there exists a single population of microcysts, lined by epithelium of apocrine type and containing fluid with a high content of K+ and DHA sulphate. This is in contrast to the two populations of macrocysts which are lined by either apocrine or flattened epithelium and differ in electrolyte and DHA sulphate content. As microcysts are considered to be the precursors of macrocysts (Wellings 1980, Haagensen et al 1981, Schwartz 1983), these two populations of macrocysts must arise from the single population of microcysts. It should be stressed that microcysts should be clearly distinguished from blunt ducts and they occur in breasts at a greater frequency than macrocysts (Azzopardi 1979, Haagensen et al 1981, Schwartz 1983). Preliminary studies suggest that multiple cysts in any one patient are usually all of the same type, ie all apocrine or all flattened. There may therefore be factors which determine whether microcysts enlarge and, if they do so, whether, in any individual, they develop into apocrine or flattened macrocysts.

The finding of high K+ and DHA sulphate concentrations in microcyst fluid suggests that these cannot arise by simple lobular dilatation due to a build up of breast secretions, as the latter contain much lower levels of K+ and DHA sulphate. This is supported by the finding of DHA sulphate in the cytoplasm of the epithelium lining microcysts but not in the cytoplasm of normal lobular epithelium. It is therefore unlikely that any microcysts arise by simple lobular

dilatation (Wellings 1980). The essential change in the formation of a microcyst would appear to be the development of apocrine change within the lobular unit. Other workers have identified microclusters of apocrine epithelium within lobules in breasts containing microcysts and have confirmed that microcysts are lined by uniform apocrine epithelium (Vilanova et al 1983). The active apocrine epithelium may lead to production of larger amounts of fluid than normal. The gelatinous nature of microcyst fluid may account for why it does not drain into the terminal duct. An alternative reason for the build-up of fluid is that there may be some obstruction to the outflow, one possible cause being the hyperplasia that is a frequent accompaniement of microcysts. However, the inability despite detailed study to find a lesion causing obstruction in the majority of both micro and macrocysts suggests that it may not be a necessary or important factor in the aetiology and development of breast cysts.

This study therefore supports the view that cysts arise in lobules. It suggests that apocrine secretory activity of the lobule is the essential feature for cyst development. Only one population of microcysts has been identified and, as these enlarge, they can maintain their secretory active apocrine epithelium and to do this there must be an increase in the number of cells lining the cyst by hyperplasia or the epithelium may become flattened and attenuated with little increase in cell numbers. This indicates that all epithelium lining cysts originates from apocrine epithelium and would explain why all cyst epithelium stains to some degree with

the apocrine marker, Gross Cystic Disease Fluid Protein 15 (Mazoujian et al 1983). What factors determine whether microcysts increase in size and whether they retain their active apocrine epithelium as they enlarge remains uncertain.

HUMAN BREAST CARCINOMAS

Apocrine Differentiation in Human Breast Carcinomas

Introduction

It is of interest in view of the finding that cysts lined by apocrine epithelium are associated with an increased frequency of hyperplasia and breast carcinoma, that certain carcinomas show apocrine differentiation (Azzopardi 1979). It has long been recognised that malignant change may occur in apocrine epithelium (Borst 1904, Berka 1911, Krompecher 1913, Cheatle et al 1931, Lee et al 1933, Ewing 1940, Stewart 1950, Bonser et al 1961). The epithelium may be lining a breast macrocyst and Bonser et al (1961) claimed that in 12 of a series of 220 unselected carcinomas arose in cysts lined by apocrine epithelium. However, origin of a carcinoma in an apocrine cyst does not automatically mean that the carcinoma will show apocrine differentiation (Azzopardi 1979). An alternative theory to explain apocrine differentiation in breast carcinomas is that apocrine change takes place pari passu with neoplasia (Azzopardi 1979).

The incidence of apocrine differentiation in reported series varies according to the definition accepted by the authors. It is generally accepted that between 2 and 15% of all carcinomas show marked apocrine differentiation (Geschickter 1943, Bonser et al 1961, Fisher et al 1975, Azzopardi 1979, Dixon et al 1983). These figures contrast with the 42-68% recorded by Haagensen et al (1981).

A classification of carcinomas into 3 groups based on degree of apocrine differentiation has been suggested (Dixon et al 1983). The features shown in Table I, which are accepted as characteristic of apocrine epithelium, were assessed as present to a marked degree, present to a moderate degree or absent and Figures 1, 2, 3 and 4 show examples of these three groups of tumours. Overall 12% showed a marked, 63% a moderate and 25% showed no features of apocrine differentiation. The figure of 12% with marked apocrine characteristics is similar to that previously reported and the 75% showing apocrine differentiation to any degree correlates with the incidence reported by Haagensen et al (1981).

In this thesis, it has been shown that both apocrine derived secretion and the cytolasm of apocrine epithelium contain high concentrations of DHA sulphate. In view of the high concentrations of androgens associated with apocrine epithelium, it is of interest that tumours with marked apocrine differentiation have been reported to metabolise significantly more testosterone in vitro than those with moderate or absent apocrine features (Dixon et al 1983). This metabolism was particularly to highly active androgens. It thus appears that the histological feature of apocrine differentiation is associated with differences in the handling of androgens and differences in biological activity.

cytoplasm :	copious, acidophilic, granular
glandular margins:	convex, bulbous, apical snouts
nuclei:	vesicular with prominent nucleoli

Table I : Features of apocrine epithelium used to assess degree of apocrine differentation in human breast cancers.

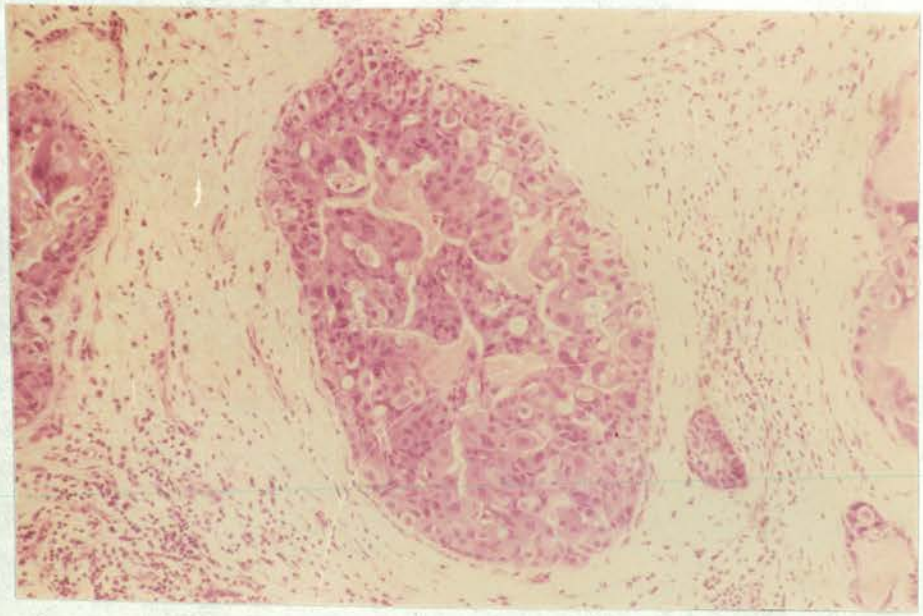


Figure 1

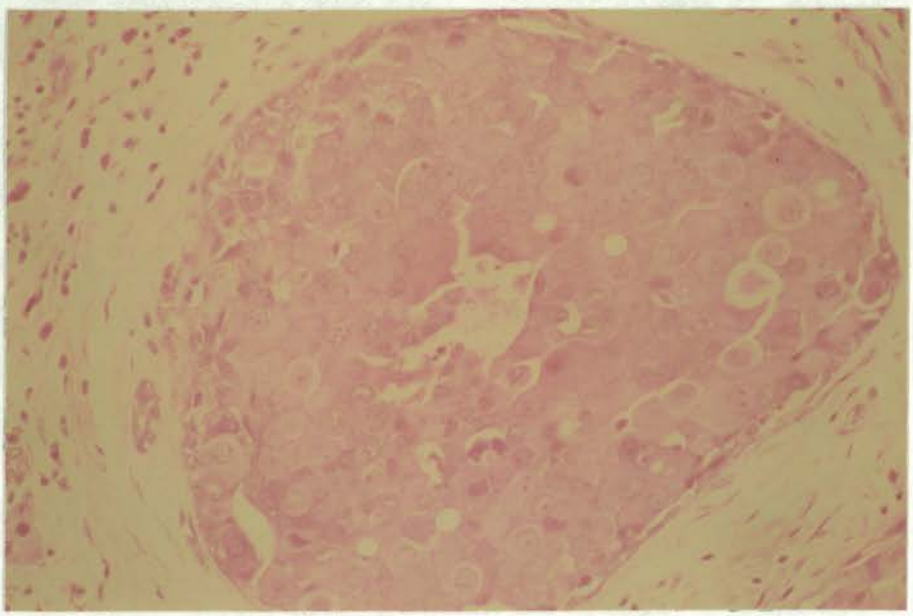


Figure 2

Breast carcinoma showing a marked degree of apocrine differentiation (H & E x 100 (Figure 1) and x 400 (Figure 2))

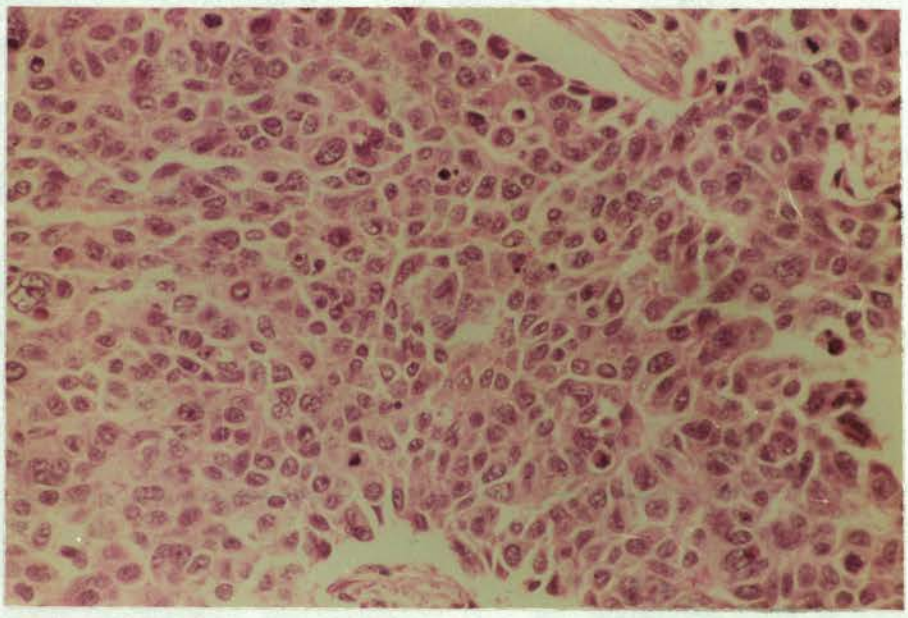


Figure 3 Breast carcinoma showing a moderate degree of apocrine differentiation (H & E x 100)

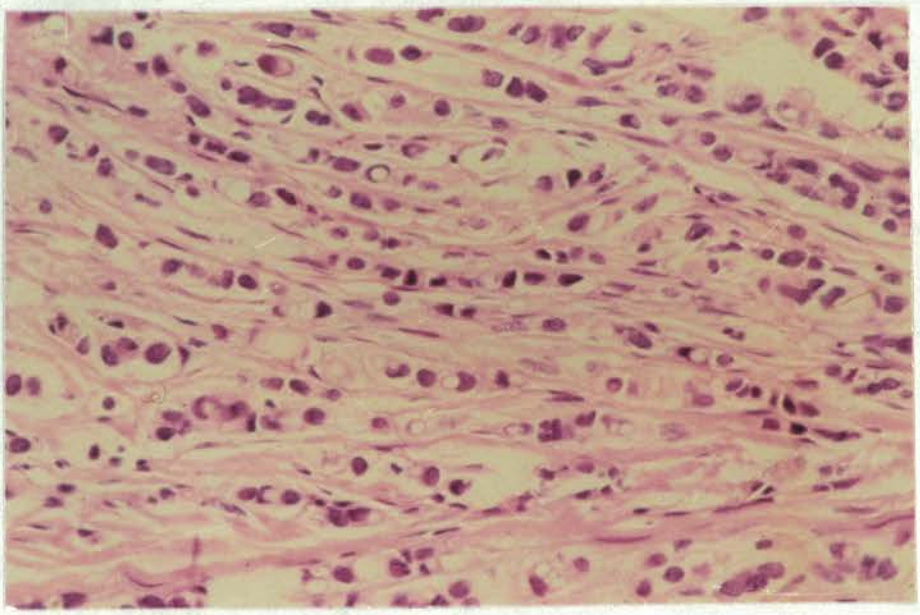


Figure 4 Breast carcinoma having no apocrine features (H & E x 100)

Apocrine differentiation in human breast carcinomas

(i) Demonstration of DHA sulphate in human breast carcinomas by the immunoperoxidase technique

(ii) Correlation of DHA sulphate in human breast carcinomas with the degree of apocrine differentiation and oestrogen receptor content

(iii) Electron microscopy of tumours with marked apocrine differentiation

Demonstration of DHA sulphate in human breast carcinomas by the immunoperoxidase technique

The aim of the initial study was to determine whether DHA sulphate could be located in human breast carcinomas.

Materials and Methods

Frozen sections were cut from 20 human breast cancers and DHA sulphate was localised in the tumours by the immunoperoxidase technique. The amount of staining within the tumour was scored as ++, + or absent.

Results

Of the 20 carcinomas, 5 showed marked amounts of material (++) cross-reacting with the antibody, 8 showed minimal staining (+) and 8 showed no staining. Examples of ++, + and absent staining are shown in Figures 5, 6 and 7. Staining was abolished by pre-incubation of the primary antibody with DHA sulphate (Figures 8a and b). One carcinoma was seen to arise in an apocrine cyst and staining was present in both benign and malignant areas of the cyst.

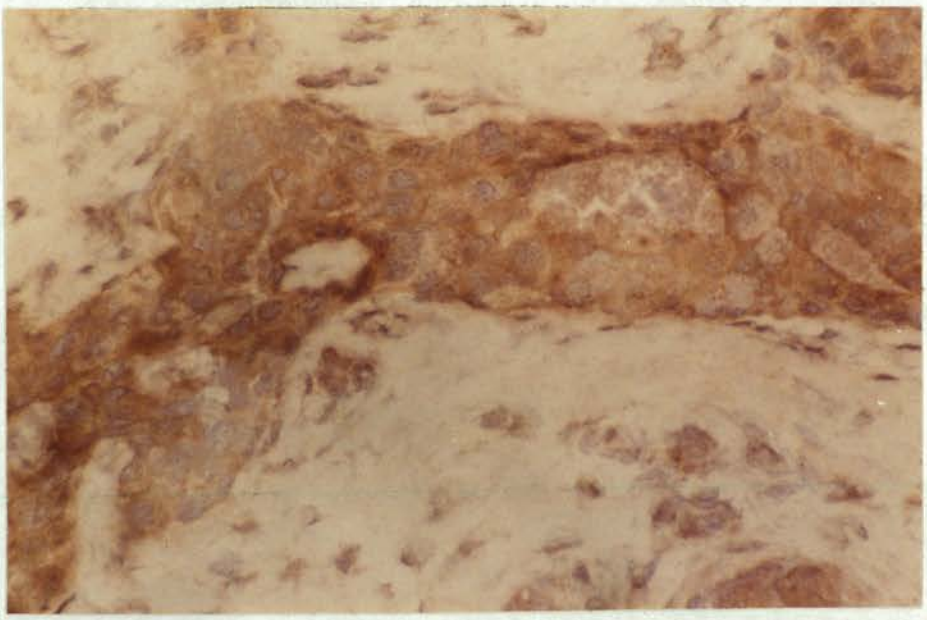


Figure 5 Breast carcinoma with a marked apocrine differentiation stained to demonstrate the presence of DHA sulphate by the immunoperoxidase technique (frozen section x 200)

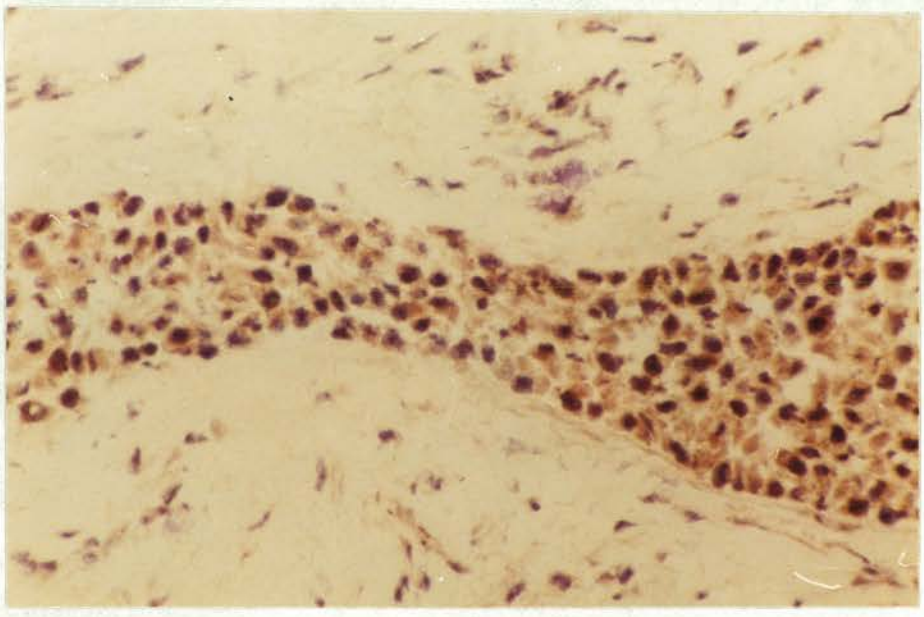


Figure 6 Breast carcinoma with a moderate degree of apocrine differentiation stained for DHA sulphate (frozen section x 200)

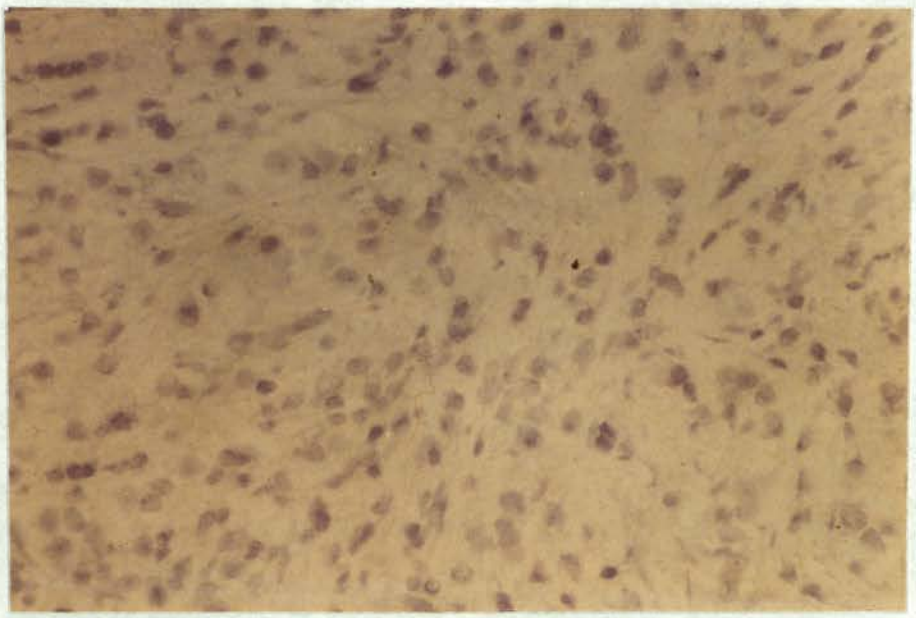


Figure 7 Breast carcinoma with no apocrine features stained for DHA sulphate (frozen section x 200)

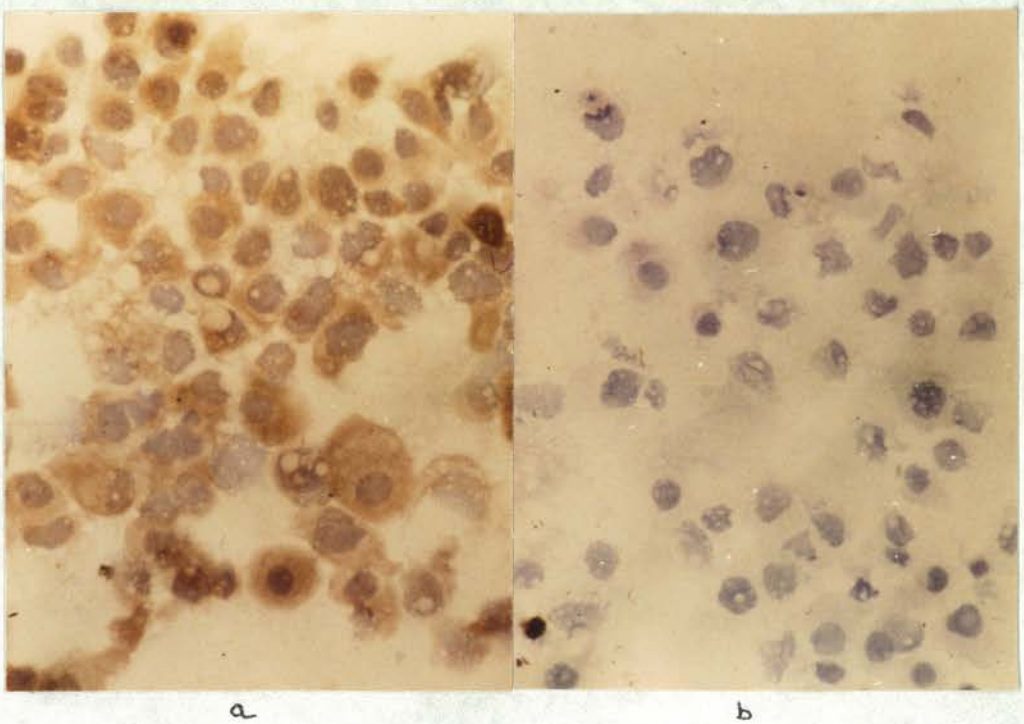


Figure 8a and b Smear from a breast carcinoma with marked apocrine differentiation stained to show DHA sulphate (a) and a negative control (b) whose primary antibody was pre-incubated with excess DHA sulphate (x 200)

Correlation of DHA sulphate in human breast carcinomas with degree of apocrine differentiation and oestrogen receptor concentration

Having identified that material is present in human breast carcinomas which cross-reacts with the antibody and is therefore probably DHA sulphate, it was hoped to determine if the amount of material present correlated with either the degree of apocrine differentiation within the tumour or the oestrogen receptor concentration.

Materials and Methods

The degree of apocrine differentiation in a tumour was scored on haematoxylin and eosin stained sections of the 20 breast carcinomas in which DHA sulphate had been localised. The tumours were graded on a 3-point scale as described earlier (marked, moderate or absent apocrine differentiation).

The oestrogen receptor concentration of each tumour was performed by Dr R A Hawkins using a saturation method (Hawkins et al 1977). All procedures were performed in a cold room. The amount of receptor present was expresed as f mol of receptor / mg tumour cytosol protein.

Correlation of the amount of DHA sulphate present as assessed by the immunoperoxidase technique and both apocrine differentiation and

oestrogen receptor concentration was by the Kendall rank correlation test.

Results

The correlation between apocrine differentiation, oestrogen receptor and degree of staining by the DHA sulphate antibody peroxidase technique is shown in Tables I and II. Apocrine differentiation was significantly correlated with degree of staining, $p < 0.01$, Kendall-Rank coefficient = 0.95. Only tumours with low oestrogen receptor content (<20 fmol/mg cytosol) protein stained to a marked degree (++) but, overall, the correlation between oestrogen receptor and degree of staining did not reach statistical significance, $p < 0.10$ Kendall rank coefficient = 0.35.

		Degree of staining with DHA sulphate antibody-peroxidase technique		
Degree of Apocrine Differentiation	Number of Tumours	++	+	0
Marked	5	4	1	0
Moderate	8	1	7	0
Absent	7	0	1	6

Table I Relationship of degree of apocrine differentiation and degree of staining with DHA sulphate antibody-peroxidase technique.

Oestrogen Receptor fmol/mg cytosol protein	Number of Tumours	Degree of staining with DHA sulphate antibody-peroxidase technique ++	+	0
0-5	7	3	2	2
5-20	6	2	3	1
>20	7	0	3	4

Table III Correlation of oestrogen receptor content and degree of staining with DHA sulphate antibody - peroxidase technique.

Electron microscopy of tumours with marked apocrine differentiation

The aim of this study was to compare and contrast the ultrastructural appearance of human breast carcinomas showing marked apocrine differentiation with benign apocrine epithelium within the breast.

Materials and Methods

Two of the five human breast carcinomas which showed marked apocrine differentiation were examined by electron microscopy.

Results

In its non-invasive form, the apocrine carcinomas had features similar to that of benign apocrine epithelium with vesicular nuclei and prominent nucleoli, cytoplasmic granules and microvilli at the glandular margins (Figure 9). The cellular contents were, however, less well organised and the nuclei larger, variable in size and more irregular. The electron dense intracytoplasmic granules seen in the subapical position in normal apocrine epithelium were still visible in the invasive tumour, but were haphazardly distributed within the cell (Figure 10), as were the many mitochondria (Figure 11), also a characteristic feature of apocrine epithelium. Budding of the apical epithelium was also identified (Figure 12) and this is not a feature of benign apocrine epithelium.

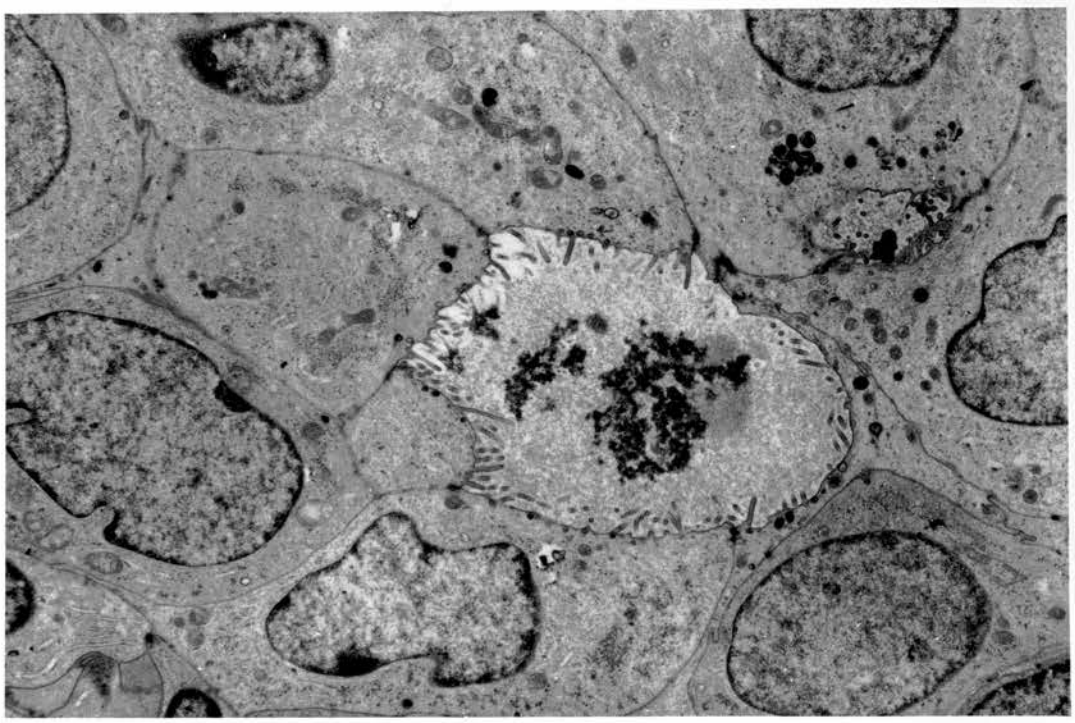


Figure 9 Electron microscopy of non-invasive 'apocrine' carcinoma. The nuclei are more irregular than those in normal apocrine epithelium but still have occasional prominent nucleoli. Dense intracytoplasmic vacuoles are seen in some cells. The cells show apical snouts and microvilli (x 6,000)

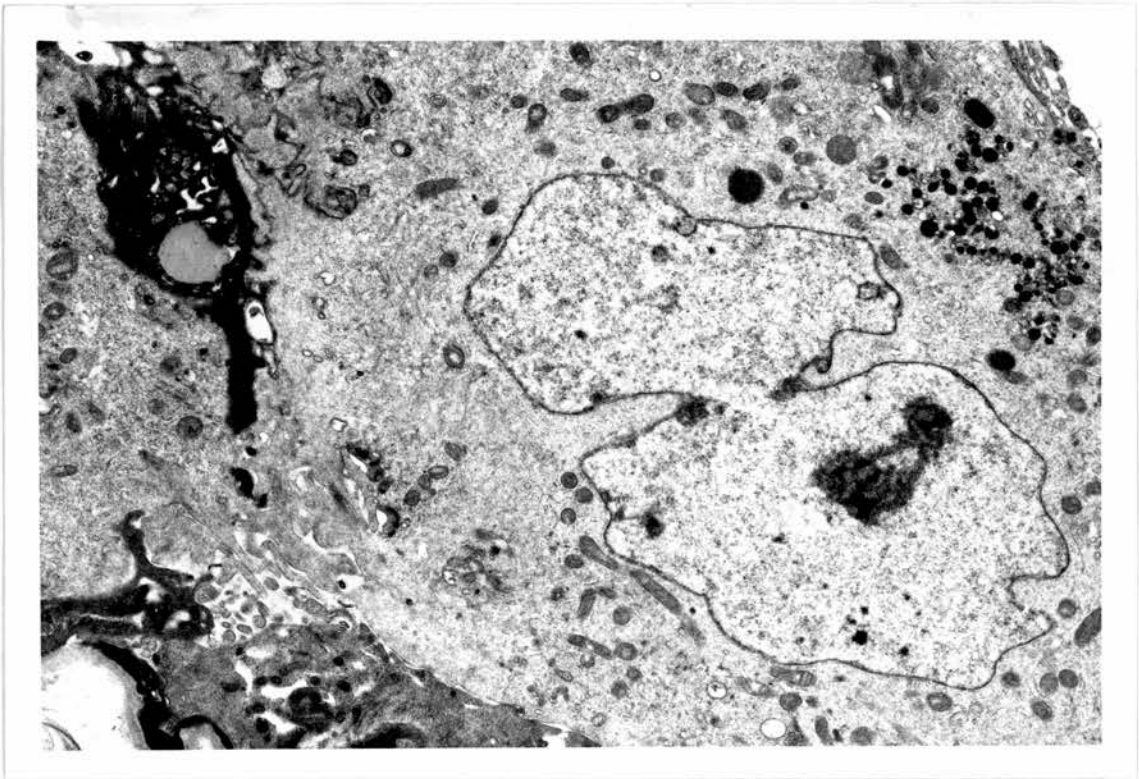


Figure 10 Electron microscopy of an invasive carcinoma with marked apocrine features. The prominent nucleolus and dense intracytoplasmic granules characteristic of apocrine epithelium are easily seen (x 8,400)

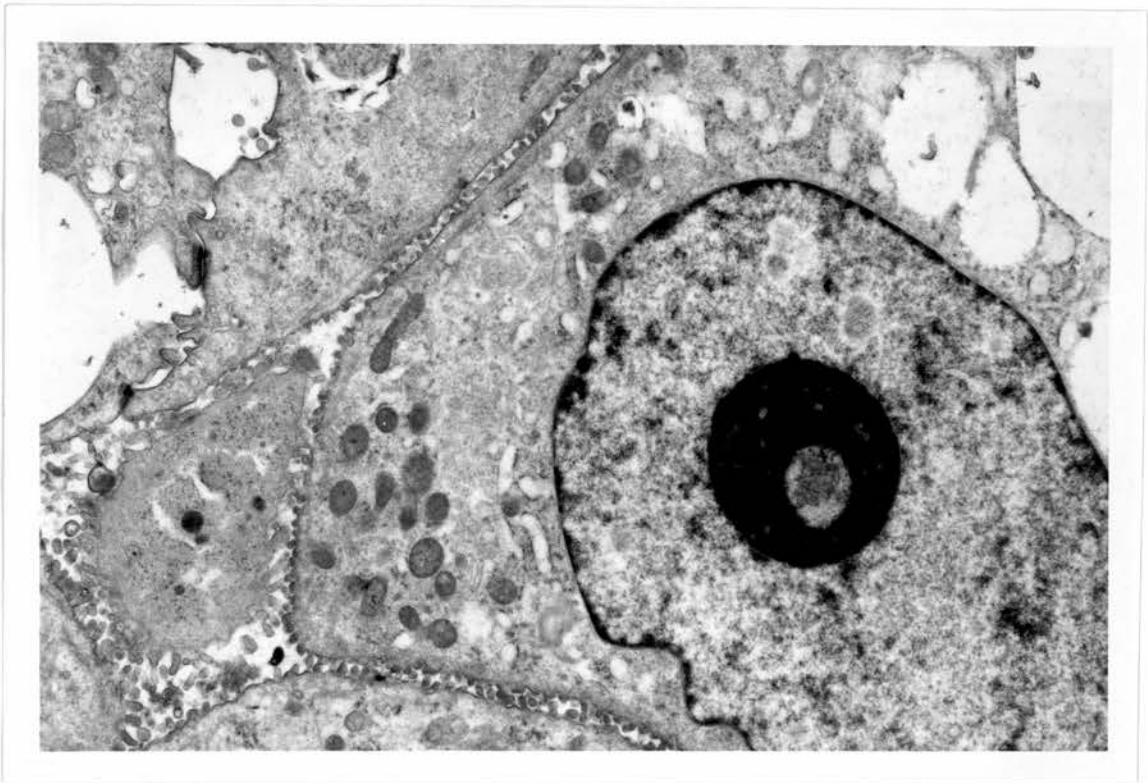


Figure 11 Further example of an invasive carcinoma with marked apocrine features (x 10,000)

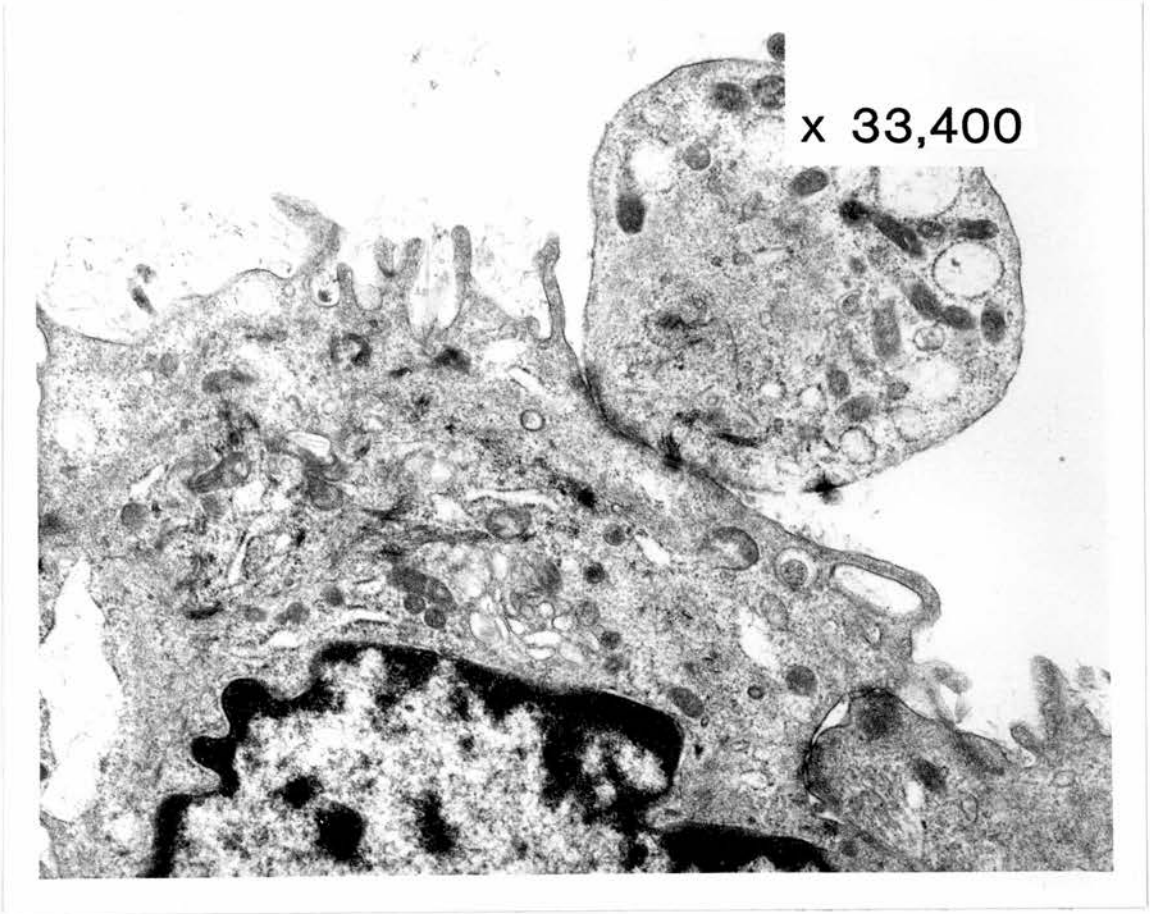


Figure 12 Budding of the cytoplasm in a carcinoma with marked apocrine features (x 33,400)

It was also evident that both benign apocrine change and carcinoma with marked apocrine features were widespread in all sections examined from each of the two breasts.

DISCUSSION

This study has shown that carcinomas which show apocrine differentiation contain high concentrations of DHA sulphate. This correlates well with the earlier observation that DHA sulphate is found both in the cytoplasm and fluid derived from benign apocrine epithelium and confirms the view that DHA sulphate is an apocrine marker. Tumours with marked apocrine differentiation have been previously shown to metabolise significantly more testosterone in vitro than tumours without these features (Dixon et al 1983). As DHA sulphate can be converted by breast tumours to testosterone (Miller et al 1976), it seems likely that high concentrations of active androgens are present within tumours with marked apocrine differentiation. Apocrine secretion is known to be androgen dependent (Hurley et al 1960) and it may be that this group of tumours are dependent on androgens. This is in keeping with these tumours being more commonly oestrogen independent, ie oestrogen receptor negative. Manipulation of androgens in such patients may therefore be of some therapeutic benefit.

The ultrastructural studies showed that apocrine carcinomas closely resemble benign apocrine epithelium in many respects. It was also evident that in breasts affected by apocrine carcinoma, there was also benign apocrine change and a range of intermediate stages of hyperplastic apocrine epithelium with and without atypia. This raises the question of whether malignancy arises in benign apocrine

epithelium or whether the same factors which institute neoplasia also cause apocrine change. Whatever the reason, it adds support to the view that breast carcinoma is more common in patients with apocrine cystic disease.

GENERAL DISCUSSION

GENERAL DISCUSSION

This study has shown that the androgen conjugate DHA sulphate is present within breast fluids and breast tissues in concentrations many times greater than those found in plasma. These observations suggest that the hormonal microenvironment of the breast varies greatly from that in plasma. Other workers have shown that other hormones are also present in breast fluids in concentrations greatly in excess of those in plasma (Raju et al 1977, Wynder et al 1977, Miller et al 1981, Bradlow et al 1983a). Despite the evidence that hormones are aetiological in breast cancer, studies of hormones in plasma and urine have failed to show consistent abnormalities in those women with breast disease. The finding that the hormonal milieu of the breast differs greatly from plasma may explain this and it is possible that the measurement of hormones in breast fluids may give more accurate information of levels within the breast than studies on plasma and urine.

The levels of DHA sulphate in breast cyst fluid have been shown to correlate directly with the K+ content and inversely with the Na+ content of the fluid. On the basis of Na+, K+ and DHA sulphate levels, two populations of cyst fluids were defined. These two populations appear to be derived from different types of epithelium, the fluids with a high DHA sulphate content (low [Na+], high [K+]) being derived from apocrine epithelium and those with a low level of DHA sulphate (high [Na+], low [K+]) being derived from flattened

epithelium. It is of interest that apocrine secretion from other sites in the body has been shown to contain a high concentration of DHA sulphate (Labows et al 1979).

The origin of DHA sulphate in the breast remains uncertain, but the finding that the concentrations of DHA sulphate in breast secretions fall after administration of agents which reduce the levels of DHA sulphate in plasma suggests that it is concentrated from plasma. This is supported by the work of Bradlow et al (1983a) who showed that labelled DHA sulphate infused intravenously is found in breast cyst fluid in levels above that in plasma. The concentration of DHA sulphate in breast tissues and fluids has been shown in this thesis to be directly related to the degree of apocrine activity; epithelium and tumours with marked apocrine differentiation being associated with the presence of large amounts of this androgen conjugate. DHA sulphate may therefore be a marker of apocrine activity and its presence in the breast is in keeping with the view that the breast is merely a modified apocrine gland (Petrakis 1977a).

Apocrine activity of the breast lobular epithelium may be of importance in the evolution of breast cysts as the present study has shown that all breast microcysts are lined by apocrine type epithelium and contain high concentrations of DHA sulphate. This activity appears to be a pre-requisite to the development of microcysts (Vilanova et al 1983). What factors determine the degree of apocrine activity within the breast remain uncertain, but it is

known that apocrine glands at other sites of the body are under partial control by androgens (Hurley et al 1960, Labows et al 1976). As has previously been noted, the frequency of cysts is reduced by the oral contraceptive pill (Pastides et al 1983) which reduces apocrine activity in other apocrine glands (Royal College of General Practitioners 1974) and also by cyproterone acetate, an antiandrogenic compound.

DHA sulphate itself has little biological activity. It can, however, be converted within the breast to a variety of other steroids including more active androgens and oestrogens (Abul-Hajj 1975, Miller et al 1979, Miller et al 1984). It has been suggested, for instance, that the other steroid hormones present in cyst fluids are derived from DHA sulphate (Bradlow et al 1981a, 1983a). An important finding has been that there are differences in behaviour in cyst fluids which differ in their concentration of DHA sulphate. Patients with cysts lined by apocrine epithelium, which contain high levels of DHA sulphate, are many times more likely to develop further cysts and may be at a greater risk of breast cancer than those patients with cysts lined by flattened epithelium. How this correlates with the finding of Bulbrook et al (1976) that it is women with reduced urinary excretion of androgen metabolites who are at increased risk of breast cancer is uncertain, although this study has clearly shown that there is little direct relationship between plasma, urine and breast levels of steroids and has therefore questioned the relevance of measurements of steroids in urine or plasma. The measurement of DHA sulphate in breast fluids appears to

provide a method of assessing the degree of apocrine activity within the breast. As apocrine activity appears related to the development of both benign (Azzopardi 1979, Schuerch et al 1982, Vilanova et al 1983) and malignant breast disease (Azzopardi 1979, Haagensen et al 1981, Schuerch et al 1982), further studies of this androgen conjugate in breast fluids are warranted.

BIBLIOGRAPHY

REFERENCES

ABUL-HAJJ YJ (1975). Metabolism of dehydroepiandrosterone by hormone independent human breast carcinoma. Steroids 24: 488.

ADAIR FE and PACK GT (1931). The use of electric breast pumps as a prophylactic measure against secretory stagnation in the breast and possibly mammary cancer. Ann Surg 94: 154.

ADAMS JB and LI K (1975). Biosynthesis of 17β oestradiol in human breast carcinoma tissue and a novel method for its characterisation. Br J Cancer 31: 429.

AHMED A (1975). Apocrine metaplasia in cystic hyperplastic mastopathy: histochemical and ultrastructural observations. J Path 115: 211.

AHMED A (1978). Atlas of the ultrastructure of human breast disease. Churchill Livingstone, Edinburgh 41.

AZZOPARDI JG (1979). Problems in breast pathology. Saunders, London.

BASSI F, GIUSTI E, BORSI L, CATTANED S, EIANOTT P, FORTI G, PAZZAGLI M, VIGIANI C and SERIO M (1977). Plasma androgens in women with hyperprolactinaemic amenorrhoea. Clin Endocrinol 6: 5.

BERKA F (1911). Die Brustdruse verschiedener Altersstufen und watrend der Schwangershaft. FrankF z F Path 8: 203.

BERTA L, FRAIRIA R, BUMMA C, NAVELLO C, MOSETTI C, ROVERO E and GAIDANO G (1983). Hormonal pattern and sex hormone binding globulin in plasma of women with cystic disease - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York 123.

BLACK MM, BARCLAY TCM, CUTLER SJ, HANKEY BF and ASIRE AJ (1972). Association of atypical characteristics of benign breast lesions with subsequent risk of breast cancer. Cancer 29: 338.

BLOODGOOD JC (1906). Senile parenchymatous hypertrophy of the female breast: its relation to cyst formation and carcinoma. Surg Gynecol Obstet 3: 721.

BOCUZZI G, BRIGNARDELLO E, AGRIMONTI F, DOGLIOTTI L and ANGELI A (1983). Adrenal androgens and prolactin in human breast cyst fluid: a possible correlation - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York 85.

BOLANDER FF and TOPPER YJ (1980). Stimulation of lactose synthetase activity and casein synthesis in mouse mammary explants by estradiol. Endocrinology 106: 407.

BONSER GM, DOSSETT JA and JULL JW (1961). Human and experimental breast cancer. Pitman, London.

BORST T (1904). Ueber atypische Epithelwocherungenand Krebse. Verh dtsch path Ges 7: 110.

BOSTON COLLABORATIVE DRUG SURVEILLANCE PROGRAMME (1973). Oral contraceptives and venous thromboembolic disease, surgically confirmed gallbladder disease and breast tumours. Lancet (i): 1399.

BRADLOW HL, FUKUSHIMA DK, ROSENFELD RS, BOYAR RM, KREAM J, FLEISHER M and SCHWARTZ MK (1976). Hormone levels in breast cyst fluid. Clin Chem 22: 1213.

BRADLOW HL, SCHWARTZ MK, FLEISHER M, NISSELBAUM JS, BOYAR R, O'CONNOR J and FUKUSHIMA DK (1979). Accumulation of hormones in breast cyst fluid. J Clin Endocrinol Metab 49s: 778.

BRADLOW HL, ROSENFELD RS, KREAM J, FLEISHER M, O'CONNOR J and SCHWARTZ MK (1981a). Steroid hormone accumulation in human breast cyst fluids. Cancer Res 41: 105.

BRADLOW HL, SKIDMORE FD, SCHWARTZ MK and FLEISHER M (1981b). Cation levels in human breast cyst fluid. Clin Oncol 7: 388.

BRADLOW HL, SCHWARTZ MK, FLEISHER M, ROSENFELD RS, KREAM J, SCHWARTZ D, BREED CN and FRACCHIA AA (1983a). Hormone levels in human breast cyst fluid - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York 59.

BRADLOW HL, SKIDMORE FD, SCHWARTZ MK, FLEISHER M and SCHWARTZ D (1983b). Cations in breast cyst fluid - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York 197.

BRISSAUD E (1884). Anatomie pathologique de la maladie kystique des mammelles. Arch Physiol Norm Pathol 3: 98.

BRODIE BC (1846). Lectures in Pathology and Surgery. Longmans, London.

BULBROOK RD and HAYWARD JL (1976). Abnormal urinary steroid excretion and subsequent breast cancer. Lancet (i): 519.

BULBROOK RD, MOORE JW, CLARK GMG, WANG DY, TONG D and HAYWARD JL (1978). Plasma oestradiol and progesterone levels in women with varying degrees of risk of breast cancer. Eur J Cancer 14: 1369.

BUSTER JE and ABRAHAM GE (1972). Radioimmunoassay of plasma dehydroepiandrosterone sulphate. Anal lett 5: 543.

CHEATLE GL and CUTLER M (1931). Tumours of the breast. Arnold, London.

CHETTY U (1983). Personal communication.

COOPER AP (1829). Diseases of the Breast, Part I. Longmans, London.

COWIE AT, FORSYTH IA and HART IC (1980). Endocrine control of mammary gland growth in hormonal control of lactation. Monograph Endocrinol 15: 1

CREIGHTON C (1902). Cancers and other tumours of the breast. Williams and Norgate, London.

DAVIS MM, SIMONS M and DAVIS JB (1964). Cystic disease of the breast: relationship to carcinoma. Cancer 17: 957.

DAWSON EK (1932). Sweat gland carcinoma of the breast. Edinb Med J 39: 409.

DAWSON EK (1933). Carcinoma of the mammary lobule and its origin. Edinb Med J 40: 57.

DIXON JM, SHIVAS AA, MILLER WR, TELFORD J and FORREST APM (1983). Metabolism of 7α ^{3}H testosterone in human breast cancers compared with degree of apocrine differentiation. Br J Surg 70: 685.

DONNELLY PK, BAKER KW, CARNEY JA and O'FALLON WM (1975). Benign breast lesions and subsequent breast carcinoma in Rochester, Minnesota. Mayo Clinic Proceedings 50: 650.

EWING J (1940). Neoplastic Diseases. Saunders, Philadelphia.

FASAL E and PAFFENBARGER RS Jr (1975). Oral contraceptives as related to cancer and benign lesions of the breast. J Natl Cancer Inst 55: 767.

FISHER ER, GREGORIO RM and FISHER B (1975). The pathology of invasive breast cancer. Cancer 36: 1.

FLEISHER M, OETTGEN MF, BREED CN, ROBBINS GF, PINSKY CM and SCHWARTZ MK (1974). CEA-like material in fluid from benign cysts from the breast. Clin Chem 20: 41.

FOOTE FW and STEWART FW (1945). Comparative studies of cancerous and non-cancerous breasts. Ann Surg 121: 6.

FRANTZ VK, PICKREN JW, MELCHER GW and AUCHINLOSS H Jr (1951). Incidence of chronic cystic disease in so-called 'normal breasts'. Cancer 4: 769.

GAIRARD B, GROS CM, KOEHL C and RENAUD R (1983). Proteins and ionic components in breast cyst fluids - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New

York. 191

GATZY JT, ZAYLOUN MP, GASKINS K and PEARLMANN WH (1979). Electrolytes of breast cyst fluid. Clin Chem 25: 745.

GESCHICKTER CF (1943). Diseases of the Breast. Lippincott, Philadelphia.

GOLINGER RC (1978). Hormones and the pathophysiology of fibrocystic mastopathy. Surg Gynecol Obstet 146: 273.

GUIST G, BASSI F, BORSI L, CATTANEO S, GIANOTTI P, LANZ L, PAZZOGLI M, VIRGIANI C and SERIO M (1977). Effects of prolactin on the human adrenal cortex: plasma dehydroepiandrosterone sulphate in women affected by amenorrhoea with hyperprolactinaemia - in Prolactin and Human Reproduction. Ed Crosignavi PG and Robyn C, Academic Press, New York. 239

HAAGENSEN CD (1971). Diseases of the Breast. Saunders, Philadelphia.

HAAGENSEN CD, BODIAN C and HAAGENSEN DE (1981). Breast carcinoma risk and detection. Saunders, Philadelphia.

HAAGENSEN DE Jr, MAZOUJIAN G, HOLDER WR Jr, KISTER SJ and WELLS SA Jr (1977). Evaluation of a breast cyst fluid protein detectable in the plasma of breast carcinoma patients. Ann Surg 185: 279.

HAAGENSEN DE Jr, MAZOUJIAN G, DILLEY WG, PEDERSON CE, KISTER SJ and WELLS SA Jr (1979). Breast gross cystic disease fluid analysis. I Isolation and radioimmunoassay for a major component protein. J Natl Cancer Inst 62: 239.

HAAGENSEN DE Jr (1983). Personal communication.

HURLEY HJ and SHELLEY WB (1961). The human apocrine sweat gland in health and disease. Thomas, Springfield USA.

IZUO M, OKAGAKI T, RICHART MM and WATTES R (1971a). DNA content in 'apocrine metaplasia' of fibrocystic disease of the breast. Cancer 27: 643.

IZUO M, OKAGAKI T, RICHART MM and CATTES R (1971b). Nuclear DNA content in hyperplastic lesions of cystic disease of the breast with special reference to malignant alteration. Cancer 28: 620.

JACKSON D, TODD DA and GORSUCH PL (1951). Study of breast secretion for detection of intramammary pathologic change and of silent papilloma. J Int College Surg 15: 522.

KALACHE A and VESSEY M (1982). Risk factors in breast cancer in Clinics in Oncology. Ed Baum M, Sanders, London 1: 661.

KELSEY JL, LINDFORS KK and WHITE C (1974). A case control study of the epidemiology of benign breast disease with reference to oral contraceptive use. Int J Epidemiol 228: 551.

KERN WN and BROOKS RN (1969). Atypical epithelial hyperplasia associated with breast cancer and fibrocystic disease. Cancer 24: 668.

KÖNIG F (1893). Mastitis chronica cystica. Kentralbl Chir 20: 49.

KORENMAN SG (1980a). The endocrinology of breast cancer. Cancer 46: 874.

KORENMAN SG (1980b). Oestrogen window hypothesis of the aetiology of breast cancer. Lancet (ii): 700.

KROMPECHER E (1913). Uber Schweissdrusserzysten der Brustdrusse und deren Krebse. Dtch Path 16: 365

KULSKI JK, SMITH M and HARTMAN PE (1977). Perinatal concentration of progesterone, lactose and lactalbumin in the mammary secretion of women. J Endocrinol 74: 509.

KUTTENN F, FOURNIER S, SITRUK-WARE R, MARTIN P and MAUVAIS-JARVIS P (1983). Progesterone insufficiency in benign breast disease - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York.231

LABOWS JN, PRETI G, HOELZLE E, LEYDEN J and KLIGMAN A (1979). Steroid analysis of human apocrine secretion. Steroids 34: 249.

LEE BJ, PAGE GT AND SCHNARGEL I (1933). Sweat gland cancer of the breast. Surg Gynecol Obstet 56: 975.

LEE AW, BURNS PE and GRACE M (1978). Oral contraceptives and breast disease in premenopausal northern Alberta women. Int J Cancer 22: 700.

MARCHESONI D, GANGEMI M, MOZZANEGA B, NARDELLI GB, MAGGINO T and VELASLO M (1983). Hormonal investigations in benign breast disease - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York 145.

MASON RC (1981). Human breast cancer: drugs, circulating plasma hormones and hormone binding proteins, tumour steroid receptors and paraendocrine potential. MD Thesis, Victoria, University of Manchester.

MAUVAIS-JARVIS P and KUTTENN F (1975). L'insufficience en progesterone, est elle cancerigene? Nouv Presse Med 4: 323.

MAUVAIS-JARVIS P, SITRUK-WARE R, KUTTENN F and STERKERS N (1979). Luteal phase insufficiency: a common pathophysiological factor in development of benign and malignant breast disease - in Commentaries on Research in Breast Disease. Ed Bulbrook RD and Taylor DJ, Liss, New York. 25

MAZOUJIAN G, PINKUS G, DAVIS G and HAAGENSEN DR Jr (1983). Immunohistochemistry of a gross cystic disease fluid protein (GCDFP15) of the breast. Am J Pathol 110: 105.

MELIS GB, GUARNIERI G, PAOLETTI AM, PTACCHI FD, RICCI G, STRIGINI F, SELLI M, RUJU A and FIORETTI P (1983). Clinical significance of hormonal evaluation in peripheral blood and in breast cyst fluid of women with benign breast disease - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York. 101

MILLER AB and BULBROOK RD (1980). The epidemiology and etiology of breast cancer. N Eng J Med 303: 1246.

MILLER WR and FORREST APM (1976). Oestradiol synthesis from C19 steroids by human breast cancers. Br J Cancer 33: 116.

MILLER WR, HAWKINS RA, CREEL RJ and FORREST APM (1977). Oestrogen in breast fluid. Lancet (ii): 1179.

MILLER WR, HUMENIUK V and KELLY RW (1980). Dehydroepiandrosterone sulphate in breast secretions. J Steroid Biochem 13: 145.

MILLER WR, HUMENIUK V AND FORREST APM (1981). Factors affecting dehydroepiandrosterone sulphate levels in human breast secretions. Breast Cancer Res Treat 1: 267.

MILLER WR, ROBERTS MM, CREEL RJ, YAP PL, KELLY RW and FORREST APM (1982). Androgen conjugates in human breast cyst fluids. J Natl Cancer Inst 69: 1055.

MILLER WR and FORREST APM (1983). Androgen conjugates in human breast secretions and cyst fluids - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York.77

MILLER WR (1984). Personal communication.

MONSON RR, YEN S, MACMAHON B and WARREN S (1976). Chronic mastitis and carcinoma of the breast. Lancet (ii): 224.

MacDONALD PC, ROMBAUT PP and SHTERI P (1967). Plasma precursors of estrogen. I Extent of conversion of plasma $\Delta 4$ androstenedione to estrone in normal males and non-pregnant normal castrate and adrenalectomised females. J Clin Endocrinol Metab 27: 1103.

McFARLAND M (1922). Residual lactational acini in the female breast: their relation to chronic cystic mastitis and malignant disease. Arch Surg 5:1.

MacMAHON B, COLE P and BROWN J (1973). Etiology of breast cancer: a review. J Natl Cancer Inst 50: 21.

NICHOLSON GW (1923). Studies on tumour formation. Guy's Hosp Rep 73: 37.

ORY M, COLE P, MacMAHON B and HOOVER R (1976). Oral contraceptives and reduced risk of benign breast diseases. N Eng J Med 294: 419.

PAGE DL, VANDER-ZWAG R, ROGERS LW, WILLIAMS LT, WALKER WE and HARTMANN WM (1978). Relation between component parts of fibrocystic disease complex and breast cancer. J Natl Cancer Inst 61: 1055.

PAPANICOLAOU GN, HOLMQUIST DG and BADER GM (1958). Exfoliative cytology of the human mammary gland and its value in the diagnosis of cancer and other diseases. Cancer 11: 377.

PASTIDES M, FELSEY JL, LIVOLSI VA, HOLFORD TD, FISCHER DB and GOLDENBERG IS (1983). Oral contraceptives use and fibrocystic disease with special reference to histopathology. J Natl Cancer Inst 71: 5.

PEARLMAN WN, GUERIGUIAN JD and SAWYER ME (1973). A specific progesterone-binding component of human breast cyst fluid. J Biol Chem 248: 5736.

PETRAKIS NL, MASON L, LEE R, SUGIMUTO B, PAWSON S and CATCHPOOL F (1975). Association of race, age, menopausal status and cerumen type with breast fluid secretion in non-lactating women as determined by nipple aspiration. J Natl Cancer Inst 54: 829.

PETRAKIS NL (1977a). Breast secretory activity in non-lactating women, postpartum breast involution and the epidemiology of breast cancer. Natl Cancer Inst Monogr 47: 161.

PETRAKIS NL, DOHERTY M, LEE R, PAWSON S, HUNT TK and SCHWEIZER R (1977b). Immunoglobulin levels in breast fluids of women with breast cancer. Clin Immunopathol 7: 386.

PETRAKIS NL, MASON L, DOHERTY MA, DUPUY ME, SADEE G and WILSON CS (1977c). Effect of altering diet fat on breast fluid lipids in women. Fed Proc 36: 1163.

PETRAKIS NL, GRUENKE D, BEELEN TC, CRAIG JC and CASTAGNOL I (1978). Nicotine in breast fluid of non-lactating women. Science 19: 303.

PETRAKIS NL, MAACK CA, LEE RE and LYON M (1980). Mutagenic activity in nipple aspirates of human breast fluid. Cancer Res 40: 188.

PETRAKIS NL, GRUENKE D and CRAIG JC (1981). Cholesterol and cholesterol epoxides in nipple aspirates of human breast fluid. Cancer Res 41: 2563.

RAJU U, GANGULY M and LEVITZ M (1977). Estriol conjugates in human breast cyst fluid and in serum in premenopausal women. J Clin Endocrinol Metab 53: 847.

RECLUS P (1883). La maladie kystique des mammelles. Rev Chir 3: 761.

ROBERTS MM, JONES V, ELTON RA, FORTT RW, WILLIAMS S and GRAVELLE IH (1984). Risk of breast cancer in women with a history of benign disease of the breast. Br Med J 288: 275.

ROBYN C (1983). Endocrinological aspects of breast physiology - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York 25.

ROYAL COLLEGE OF GENERAL PRACTITIONERS (1974). Oral contraceptives and health. Pitman, London.

ROYAL COLLEGE OF GENERAL PRACTITIONERS - ORAL CONTRACEPTIVE STUDY (1977). Effect on hypertension and benign breast disease of progestogen component in combined oral contraceptives. Lancet (i): 624.

SAAR GF Von (1907). Ueber cystodenoma mammae und mastitis chronica cystica. Arch Klin Chir 84: 223.

SANDISON AT (1962). An autopsy study of the human breast. Natl Cancer Inst Monogr 8.

SANTEN RJ and WELLS SA (1980). The use of aminoglutethimide in treatment of patients with metastatic carcinoma of the breast. Cancer 46: 1064.

SARTORIUS O (1973). Breast fluid cells - help in early cancer detection. J Am Med Assoc 224: 823.

SARTWELL PE, ARTHES FG and TONASCIA JA (1973). Epidemiology of benign breast lesions: lack of association with oral contraceptive use. N Eng J Med 288: 551.

SCHIMMELBUSCH C (1890). Ueber das cystadenom dier weiblichen milchdruse. Verh Dtch Ges Chir 19: 116.

SCHUERCH C, ROSEN PP, HIRUT AT, ITABASH M, YAMOMOTO H, KINNE DW and BEATTE EJ (982). A pathologic study of benign breast diseases in Tokyo and New York. Cancer 50: 1899.

SCHWARTZ MK, FLEISHER M, BREED C, ASHIKARI R, DE PALO A, KINNE D and URBAN J (1976). Enzyme content of human breast cyst fluid. Clin Chem 223: 1213.

SCHWARTZ GF (1983). Cystic disease, a definition - in Endocrinology of Cystic Breast Disease. Ed Angeli A, Bradlow HL and Dogliotti L, Raven Press, New York 1.

SCHYVE PM, SMITHLINE F and MEUTZER MY (1978). Neuroleptic induced prolactin level elevation - an emerging issue. Arch Gen Psychiatry 35: 1291.

SEMB C (1928). Pathologico-anatomical and clinical investigations of fibro-adenomatosis cystica mammae and its relation to other pathological conditions in mamma, especially cancer. Acta Chir Scand Supp X 64: 1.

SHAPIRO S, STRASE P, VENET L and FINK R (1968). The search for risk factors in breast cancer. Ann J Public Health 58: 820.

SHERMAN BM and KORENMAN SG (1974). Inadequate corpus luteum function, a pathophysiological interpretation of human cancer epidemiology. Cancer 33: 1306.

STEWART FW (1950). Tumors of the breast - Atlas of Tumour Pathology, Section 9, Fascicle 34, Washington DC Armed Forces Institute of Pathology, 99.

STEINHOFF NG and BLACK WC (1970). Florid cystic disease preceding mammary cancer. Ann Surg 171: 501.

TIETZE F (1900). Ueber das Cystadenoma mammae (Schimmelbusch) und seine Beziehungen zum carcinom der Brustdruse. Dtsch Z Chir 56: 512.

VAN BOGAERT LJ (1976). Bases biologiques de la therapeutique des mastopathies benignes diffuses. J Gynecol Obstet Biol Reprod 5: 567.

VARELLA RM and DAO TL (1978). Estrogen synthesis and estrogen binding by human mammary tumors. Cancer Res 38: 2439.

VERMEULEN A, SUY E and RUBENS R (1977). The effect of prolactin on plasma DHEA(S) levels. J Clin Endocrinol Metab 44: 122.

VERMEULEN A and ANDO S (1978). Prolactin and adrenal androgen secretion. Clin Endocrinol 8: 295.

VESSEY MP, DOLL R and SUTTON PM (1972). Oral contraceptives and breast neoplasia: a retrospective survey. Br Med J 3: 719.

VILANOVA JR, SIMON R, ALVARES J and RIVERA-POMAR JM (1983). Early apocrine change in hyperplastic cystic disease. Histopathology 7: 693.

WADDEL WJ and BATES RG (1969). Intracellular pH. Physiol Rev 49: 285.

WARREN S (1940). The relation of "chronic mastitis" to carcinoma of the breast. Surg Gynecol Obstet 71: 257.

WELLINGS SR, JENSEN HM and MARCUM RG (1975). An atlas of subgross pathology of the human breast with reference to possible precancerous lesions. J Natl Cancer Inst 55: 231.

WELLINGS SR (1980). Development of human breast cancer. Adv Cancer Res 31: 287.

WYNDER EL and HILL HP (1977). Prolactin, oestrogen and lipid in breast fluid. Lancet (ii): 840.

WYNDER EL, HILL MP, LAAKSO L, LITTNER R and KETTUNEN K (1981). Breast secretion in Finnish women: a metabolic epidemiologic study. Cancer 47: 1444.

YAP PL, PRYDE EAD and McLELLAND DBL (1980). Milk protein concentrations in galactorrhoeic mammary secretions. J Reprod Immunol 1: 347.

YAP PL, MILLER WR, HUMENIUK V, PRYDE EAD, MIRTLE CL and McLELLAND DBL (1981). Milk protein concentrations in the mammary secretions of non-lactating women. J Reprod Immunol 3: 49.

YAP PL (1982). Biochemical studies of the normal human breast. PhD Thesis, University of Edinburgh.

YAP PL, MILLER WR, ROBERTS MM, FREEDMAN B, MIRTLE CL, PRYDE EAD and McLELLAND DBL (1983) Studies on the composition of human breast cyst fluid. Br J Cancer 46: 516.

YAP PL, MILLER WR, ROBERTS MM, CREEL RJ, FREEDMAN B, MIRTLE CL, PRYDE EAD and McLELLAND DBL (1984). Protein concentrations in fluid from gross cystic disease of the breast. Clin Oncol 10: 35.

Publications arising from this thesis

DIXON JM, MILLER WR, SCOTT WN, STEELE RJC and FORREST APM (1982). Subpopulations of human breast cysts as defined by sodium, potassium and dehydroepiandrosterone sulphate content. Langenbecks Arch Chir 357: 195.

MILLER WR, DIXON JM, SCOTT WN and FORREST APM (1983). Classification of human breast cysts according to electrolyte and androgen conjugate composition. Clin Oncol 9: 227.

DIXON JM, MILLER WR, SCOTT WN and FORREST APM (1983). Studies on breast cyst fluid. Scot Med J 28: 187.

DIXON JM, MILLER WR, SCOTT WN and FORREST APM (1983). The morphological basis of human breast cyst populations. Br J Surg 70: 604.

DIXON JM, MILLER WR, YAP PL, SCOTT WN and FORREST APM (1983). Further studies on human breast cyst fluid. Br J Surg 70: 684.

DIXON JM, MILLER WR and SCOTT WN (1984). The pH of human breast cyst fluids. Clin Oncol (in press).

DIXON JM and MILLER WR (1984). Human breast cystic disease. Breast News 1: 6.

DIXON JM, SCOTT WN and MILLER WR (1984). Predicting the natural history of breast cystic disease. Br J Surg (in press).

DIXON JM, MILLER WR, SCOTT WN and FORREST APM (1984). Natural history of human breast cystic disease: the importance of cyst type. Br J Surg (in press).

MILLER WR, DIXON JM AND FORREST APM (1984). Hormonal correlates of apocrine secretion. Ann NY Acad Science (in press).

Clinical Oncology 1983, 9, 227–232

Classification of human breast cysts according to electrolyte and androgen conjugate composition

W. R. Miller, J. M. Dixon*, W. N. Scott and A. P. M. Forrest

University Department of Clinical Surgery, Royal Infirmary, Edinburgh EH3 9YW, Scotland

(*Accepted for publication 31 March 1983*)

One hundred human breast cyst fluids have been analysed for sodium (Na^+), potassium (K^+) and dehydroepiandrosterone (DHA) sulphate. Concentrations varied greatly between individual cyst fluids, Na^+ from 20 to 185 mmol/l, K^+ from 5 to 160 mmol/l and DHA-sulphate from 1·5 to 87 μmol/l. Analysis of the inter-relationships between Na^+, K^+ and DHA sulphate revealed two major sub-populations of cyst fluids—one group in which Na^+ levels were markedly in excess of K^+ and DHA sulphate concentrations were low and the other in which K^+ was the predominant cation and DHA sulphate concentrations were high.

Introduction

In Western countries, 7% of women present with a palpable cyst in the breast (Haagensen *et al.,* 1981). Women with cystic disease are believed to have an increased risk of developing breast cancer (for reviews see Azzopardi, 1979; Haagensen *et al.,* 1981), yet comparatively little is known about the aetiology of cysts or the composition and derivation of cyst fluid.

Recent work on the composition of cyst fluid has shown that concentrations of electrolytes (Gatzy *et al.,* 1979; Bradlow *et al.,* 1981*b*) and conjugates of androgens (Bradlow *et al.,* 1981*a*; Miller *et al.,* 1982) and oestrogens (Raju *et al.,* 1977) vary widely between individual cyst fluids.

The present study describes the interrelationships between concentrations of electrolytes and androgen conjugates and suggests a classification of cyst fluids on the basis of these constituents.

Materials and methods

One hundred breast cyst fluids were obtained by needle aspiration from 85 patients. In 75 subjects a single cyst was aspirated but, in 10, multiple cysts were drained (Table 1). Volumes varied from 0·7 to 50 ml.

*J. M. Dixon is a Wellcome Surgical Research Fellow.

0305–7399/83/030227+06 $03.00/0

Table 1. Electrolyte classification of multiple cysts

Patient	Breast 1 Cyst 1	Breast 1 Cyst 2	Breast 1 Cyst 3	Breast 2 Cyst 1
1	Na^+	Na^+		
2	Na^+	Na^+	Na^+	
3	Mix	Mix		
4	K^+	K^+		
5	K^+	K^+		
6	K^+	K^+	K^+	K^+
7	K^+			K^+
8	K^+			K^+
9	K^+	K^+	Mix	
10	Na^+	K^+		Na^+

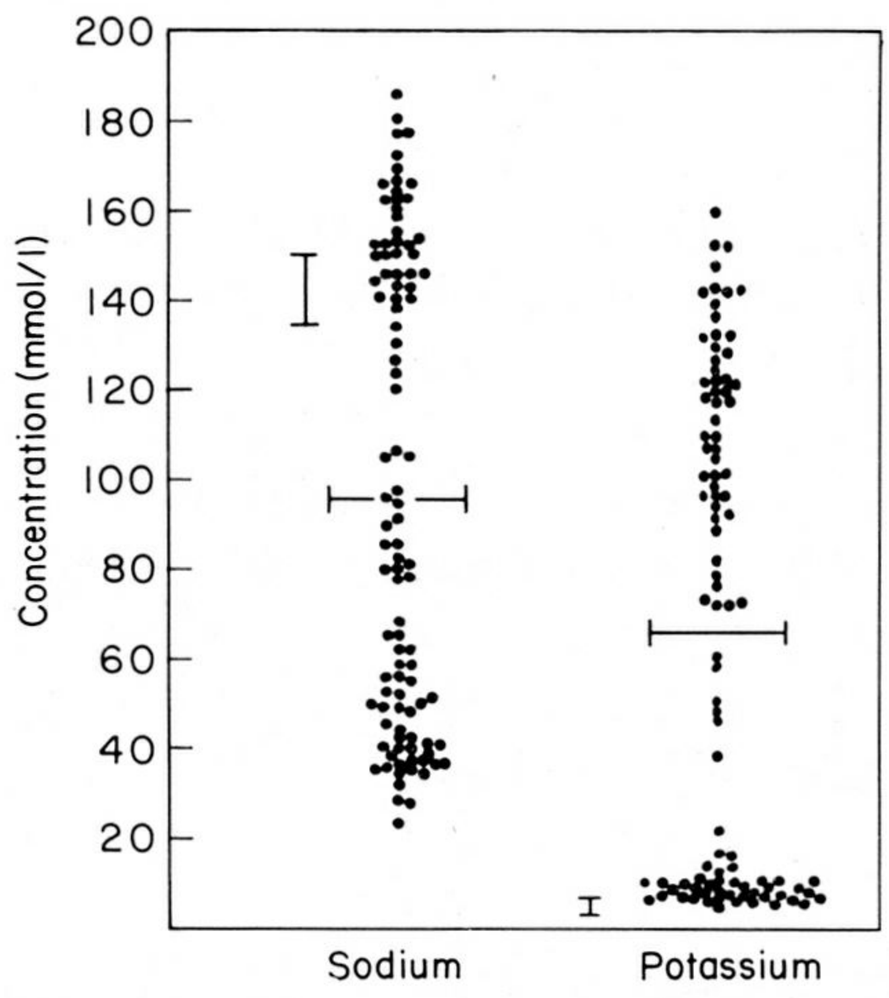


Figure 1. Concentrations of sodium and potassium in human breast cyst fluids. Horizontal lines represent mean level. Vertical lines represents reference range for human plasma.

Estimates of concentrations of Na^+ and K^+ were performed by flame photometry (EEL model 150 flame photometer) on cyst fluids diluted 1:200 in distilled water.

Androgen conjugates were measured by radioimmunoassay using an antibody described previously (Miller *et al.,* 1980). While this antibody is largely specific for dehydroepiandrosterone and its sulphate, it does cross-react with epiandrosterone conjugates. The results from the radioimmunoassay therefore reflect levels of both DHA sulphate and closely related androgen conjugates (levels of free DHA are very low in comparison with conjugated steroid). For simplicity, however, values from the radioimmunoassay have been expressed as units of 'DHA sulphate'. All statistical analyses were performed using non-parametric tests (Kendall Rank and Wilcoxon Rank tests as indicated).

Results

The levels of Na^+ and K^+ in the cyst fluids are shown in Figure 1. Values for both ions varied enormously and, in the case of potassium, were usually greatly in excess

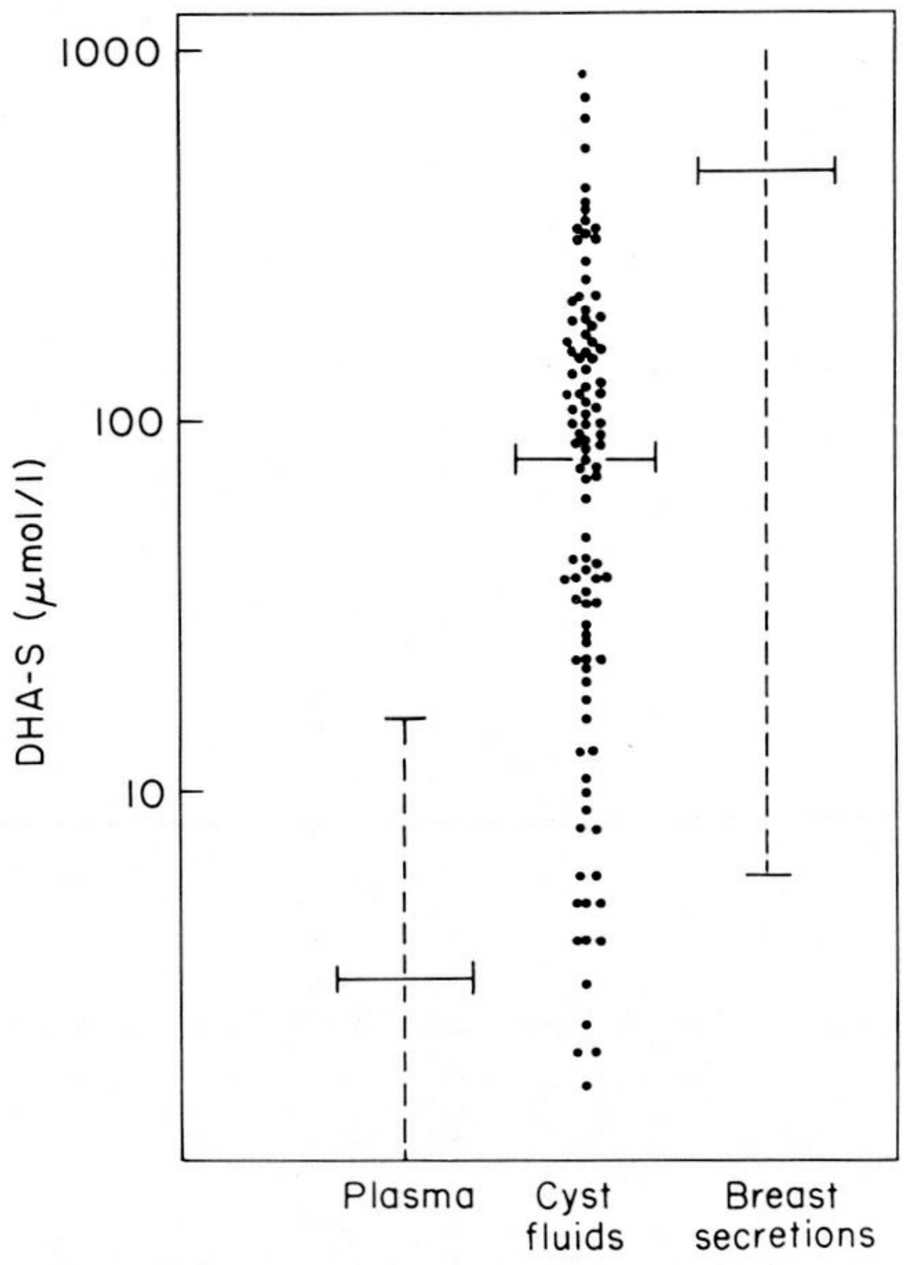


Figure 2. DHA-sulphate levels in human breast cyst fluids. Dotted vertical lines represent range in human plasma and breast secretions obtained by nipple aspiration. Horizontal lines represent median values.

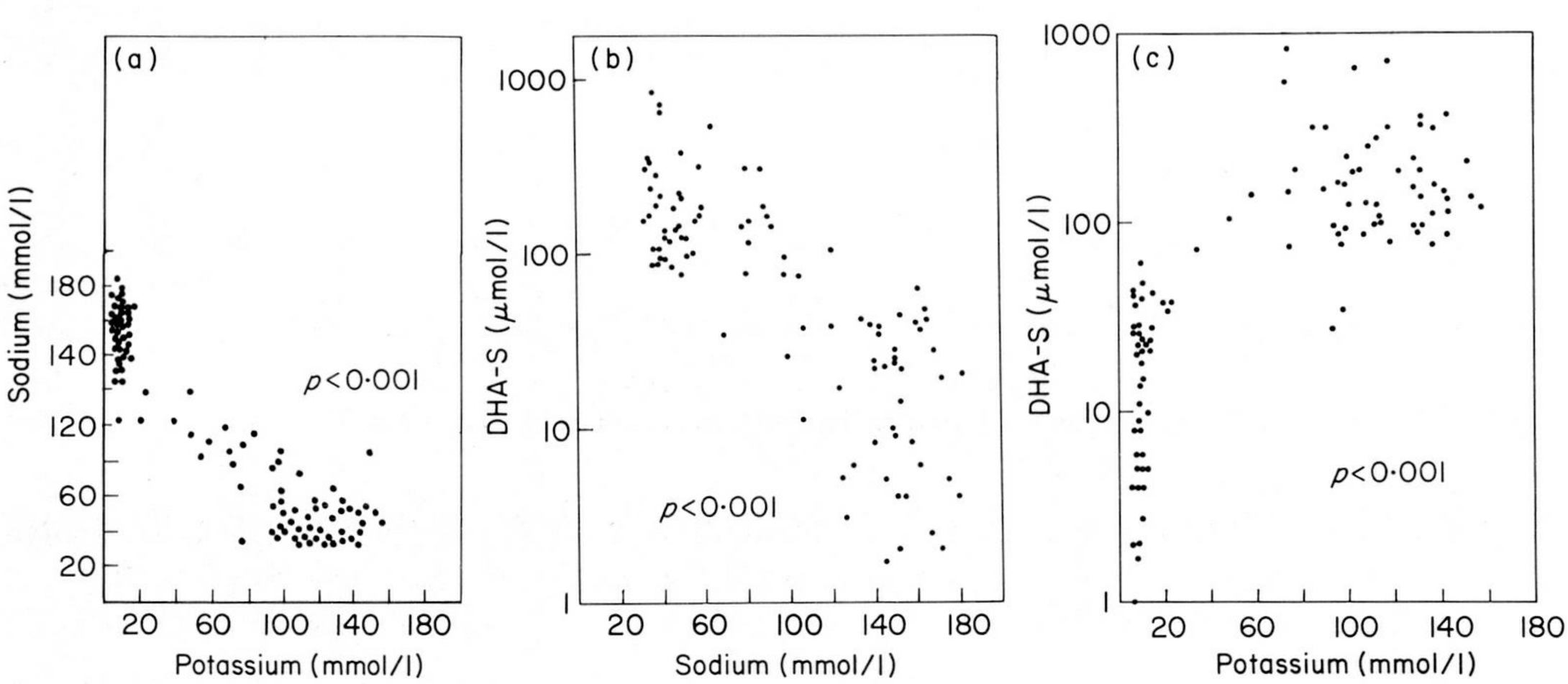


Figure 3. Relationships in human breast cyst fluid between (a) Na^+ and K^+; (b) DHA sulphate and Na^+; (c) DHA sulphate and K^+. Significance values from Kendall rank test.

of the reference range in plasma. Distribution about the mean of values for both ions was suggestive of there being more than one population of cyst fluids.

The concentrations of DHA-sulphate in each of the 100 human breast cyst fluids is shown in Figure 2. Levels varied from 1·5 to 870 μmol/l with a median value of 80 μmol/l.

Interrelationships between K^+, Na^+ and DHA-sulphate are shown in Figures 3(a), (b) and (c). These were all statistically significant by the Kendall Rank test ($p < 0 \cdot 001$ in each case). The correlations between Na^+ and both K^+ and DHA sulphate were

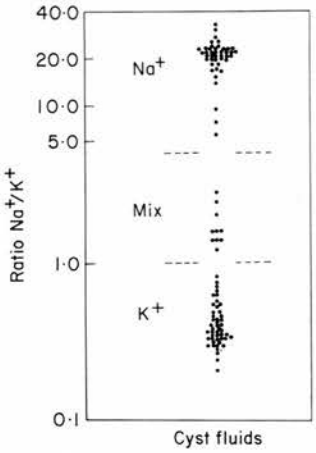


Figure 4. Ratio of Na^+/K^+ in human breast cyst fluids. Lines represent arbitrary divisions to give Na^+ (ratio >4), Mix (ratio 1–4), K^+ (ratio <1).

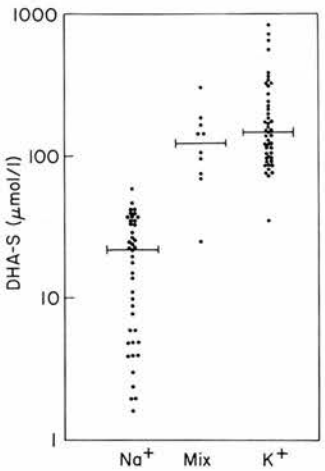


Figure 5. DHA sulphate levels in human breast cyst fluids subdivided according to electrolyte classification Na^+ represents fluids with Na^+: $K^+ > 4$, mix fluids with Na^+: K^+ 1–4 and K^+ fluids with Na^+: $K^+ < 1{\cdot}0$.

inverse relationships whereas that between K^+ and DHA sulphate was in a positive direction.

As the distribution of values for both Na^+ and K^+ suggested that there was more than one population of cyst fluids, the 100 samples examined were arbitrarily subdivided into three groups according to Na^+ and K^+ by means of their concentration ratio (Figure 4). One group was of 47 fluids in which the K^+ concentration exceeded that of Na^+ (K^+ fluids), another of 43 fluids in which the Na^+ concentration was above four-fold higher than that of K^+ (Na^+ cyst fluids), and a further group of 10 fluids with intermediate electrolyte values (mix cyst fluids). Values for DHA-sulphate in these subgroups of fluids are shown in Figure 5. Concentrations of DHA-sulphate in K^+ cyst fluids were significantly higher than the Na^+ group ($p < 0{\cdot}0005$). Levels in the mix

category were similar to the K^+ group but significantly higher than the Na^+ group ($p < 0 \cdot 001$).

Ten patients had multiple cysts aspirated on the same day. The electrolyte classification is shown on Table 1. In eight women, multiple cysts were of the same electrolyte group; two patients had cysts of different grouping although in one case the cysts co-existed within the same breast.

Discussion

Although the present study confirms that levels of Na^+, K^+ and DHA-sulphate vary widely between different cyst fluids (Bradlow *et al.,* 1981*a, b*; Miller *et al.*, 1982), it has shown that there are significant associations between the concentration of these constituents. Thus, levels of Na^+ are inversely correlated with concentrations of both K^+ and DHA-sulphate. The values for Na^+ and K^+ are also not distributed symmetrically about their mean values and distribution is suggestive of more than one population of cyst fluids. It is possible to use the relative concentrations of Na^+ and K^+ to identify presumptive sub-groups of cyst fluids. This arbitrary classification based on electrolyte composition also subdivides the cyst fluids into those with high or low levels of DHA sulphate.

It therefore can be shown that 90% of cyst fluids fall into one of two major subgroups—one type (K^+ fluids) contains high concentrations of K^+ and DHA-sulphate and low concentrations of Na^+ whereas the other type (Na^+ fluids) has the reverse composition. Bradlow and his colleagues (1981*b*) have independently classified cysts on electrolyte composition but found a higher proportion of K^+ cysts as compared with the present study.

The derivation of Na^+ and K^+ subgroups of cyst fluids still remains to be defined. They may reflect different sources of constituents or differences in secretory activity of the epithelium lining the cysts. The composition of Na^+ cysts is akin to that of plasma in respect of Na^+, K^+ and DHA-sulphate. However, although DHA-sulphate levels in K^+ cyst fluids are similar to those in breast secretions obtained by nipple aspiration (Miller *et al.,* 1981), Na^+ is the predominant ion in such breast secretions. Simple accumulation of these breast secretions is therefore unlikely to account for the formation of K^+ cyst fluids.

It seems more likely that the constituents of K^+ cyst fluids result from apocrine activity by the epithelium lining the cyst. Apocrine secretions characteristically contain high concentrations of K^+ and DHA-sulphate (Labows *et al.,* 1979). Our preliminary results examining cells lining the walls of excised cysts would support this concept.

The relevance of this classification of breast cyst fluids still remains to be elucidated and it should be noted that different types of cyst fluids may co-exist within the same breast although it is more usual for multiple cysts to be of the same type. It may be, however, that a sub-population of cysts occurs in women at high risk of epithelial proliferative changes including cancer.

References

Azzopardi, J. G. (1979). In: *Problems in Breast Pathology.* pp. 59–91. W. B. Saunders.

Bradlow, H. L., Rosenfield, R. S., Kream, J., Fleischer, M., O'Connor, J. & Schwartz, M. K. (1981*a*). Steroid hormone accumulation in human breast cyst fluids. *Cancer Research* **41**, 105–7.

Bradlow, H. L., Skidmore, F. D., Schwartz, M. K. & Fleischer, M. (1981*b*). Cation levels in human breast cyst fluid. *Clinical Oncology* **7**, 388–90.

Gatzy, J. T., Zayloun, M. P., Gaskins, K. & Pearlman, W. H. (1979). Electrolytes of breast cyst fluid. *Clinical Chemistry* **25**, 745–8.

Haagensen, C. D., Bodian, C. & Haagensen, D. E. (1981). In: *Breast Carcinoma: Risk and Detection.* pp. 55–77. W. B. Saunders.

Haagensen, D. E., Mazoajean, G., Dilley, W. G., Peterson, C. E., Kister, S. S. & Wells, S. A. (1979). Breast gross cystic disease fluid analysis I. Isolation and radioimmunoassay for a major component protein. *Journal of the National Cancer Institute* **62**, 239–46.

Labows, J. N., Preti, G., Hoelzle, E., Leyden, J. & Kligman, A. (1979). Steroid analysis of human apocrine secretion. *Steroids* **34** 249–98.

Miller, W. R., Humeniuk, V. & Forrest, A. P. M. (1981). Factors affecting dehydroepiandrosterone sulphate levels in human breast secretions. *Breast Cancer Research and Treatment* **1**, 267–72.

Miller, W. R., Humeniuk, V. & Kelly, R. W. (1980). Dehydroepiandrosterone sulphate in breast secretions. *Journal of Steroid Biochemistry* **13**, 145–51.

Miller, W. R., Roberts, M. M., Creel, R. J., Yap, P. L., Kelly, R. W. & Forrest, A. P. M. (1982). Androgen conjugates in human breast cyst fluids. *Journal of the National Cancer Institute* **69**, 1055–68.

Raju, U., Ganguly, M. & Levitz, M. (1977). Estriol conjugates in human breast cyst fluid and serum of premenopausal women. *Journal of Clinical Endocrinology and Metabolism* **45**, 429–34.

Br. J. Surg. Vol. 70 (1983) 604–606 Printed in Great Britain

The morphological basis of human breast cyst populations

J. M. DIXON, W. R. MILLER, W. N. SCOTT AND A. P. M. FORREST

University Department of Clinical Surgery, Royal Infirmary, Edinburgh EH3 9YW.

Forty human cysts have been examined to determine the relationship between the epithelial lining and the content of sodium (Na^+) and potassium (K^+) in the cyst fluid. The ratios of Na^+ to K^+ for cysts lined by flattened epithelium were higher in all cases than the values obtained for cysts lined by apocrine epithelium. These findings suggest a morphological basis for the two populations of human breast cyst fluids which can be defined on cationic content.

Human breast cyst fluids can be separated into two major populations on the basis of the relative concentrations of sodium (Na^+) and potassium (K^+) (1, 2). The two major groups are: (i) fluids in which concentrations of K^+ exceed those of Na^+; and (ii) fluids in which levels of Na^+ greatly exceed those of K^+. Cyst fluids in which K^+ is the predominant cation contain high concentrations of the androgen conjugate dehydroepiandrosterone sulphate (DHA sulphate) (2) and contain the secretory (11s) form of immunoglobulin A (IgA) (3, 4), indicating that the epithelium lining such cysts is active and secretory. In contrast cyst fluids in which Na^+ greatly predominates, contain low levels of DHA sulphate (2) and the non secretory (7s) form of IgA and would be indicative of a less active epithelium. To date, however, no evidence has been reported to show that differences in breast cyst composition are associated with the nature of the lining epithelium. The aim of the present study was to investigate the relationship between the morphology of epithelium of human breast cysts and the cationic content of cyst fluid.

Materials and methods

Morphological assessment of the epithelium lining 40 human breast cysts was performed either by: (i) centrifuging an aliquot of cyst fluid and examining the stained deposit cytologically (26 cysts from 22 patients); or (ii) dissecting cysts from tissue obtained at mastectomy or biopsy and submitting the cyst wall for histological processing and serial sectioning (7 cysts from 6 mastectomy specimens and 7 cysts from 6 biopsy specimens).

The aspirates of cyst fluid were stained by Papanicolaou's technique (Pap) and by periodic acid Schiff after diastase digestion (PAS diastase). Dissected cyst specimens were stained by haematoxylin and eosin (H & E) and PAS diastase. PAS diastase positive granules in the cytoplasm of cells were used as a positive method of identification of apocrine epithelium (5). All cyst fluid aspirates and dissection specimens were assessed by a consultant pathologist with a specific training in breast histology and cytology.

The concentration of Na^+ and K^+ in the 40 cyst fluids was measured by flame photometry (EEL model 150 flame photometer) on a 1 in 200 and a 1 in 400 dilution of cysts fluid in distilled water.

Results

It was possible to identify two major types of epithelial cells in both cytological and dissection preparations: (i) acidophilic cells (on H & E) with copious granular cytoplasm containing PAS diastase positive granules, luminal apical snouts and nuclei showing prominent nucleoli—*apocrine* epithelium; (ii) basophilic cells (on H & E) having less cytoplasm and containing no PAS diastase positive granules—*flattened* epithelium.

Figs. 1 and 2 show examples of apocrine and flattened epithelial cells in breast cyst aspirates. *Fig.* 3 shows the specific glycolipid granules in the cytoplasm of apocrine cells. *Figs.* 4 and 5 demonstrate apocrine and flattened epithelium in dissection specimens with *Fig.* 6 showing the apical position of the glycolipid granules in apocrine epithelium.

In this series no cyst showed a mixture of apocrine and flattened epithelium in either aspiration or dissection specimens. In 22 cysts the lining was assessed as apocrine (14 aspiration and 8 by dissection) and in 18 the lining was flattened simple epithelium (12 by aspiration and 6 by dissection).

The correlation between the morphology of the lining epithelium as assessed in cytological preparations and dissection specimens and the Na^+/K^+ ratio is shown in *Fig.* 7. Cysts with flattened epithelium had significantly higher Na^+/K^+ ratios than cysts lined by apocrine epithelium ($P<0{\cdot}001$ Wilcoxon's Rank Sum test). On the basis of Na^+/K^+ ratios it was possible

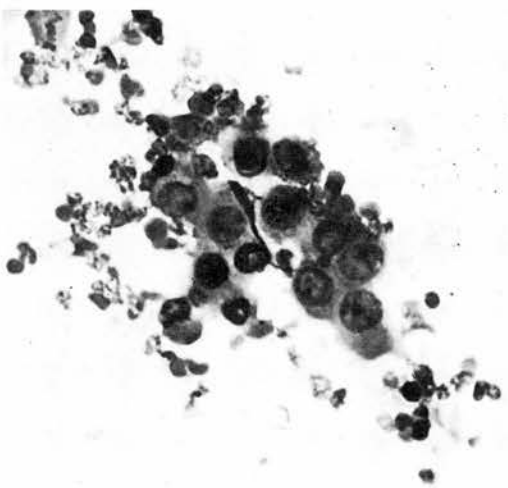


Fig. 1. *Cytological preparation of a cyst aspirate showing apocrine cells. The cells have copious pale granular cytoplasm with vesicular nuclei showing prominent nucleoli (Pap × 480).*

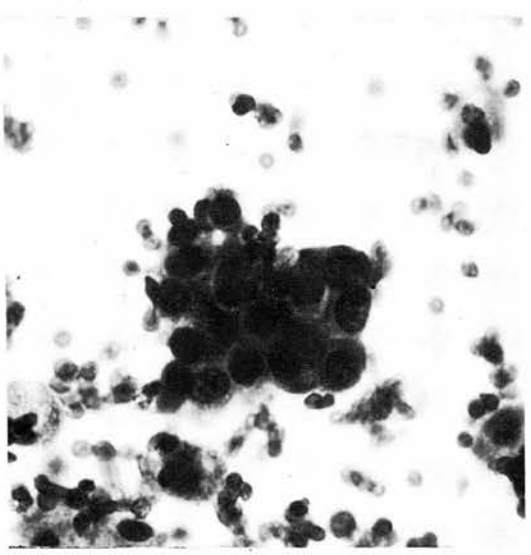


Fig. 2. *Cytological preparation of a cyst aspirate showing simple flattened epithelium. The cells have small amounts of cytoplasm, deeply staining nuclei and no special features (Pap × 480).*

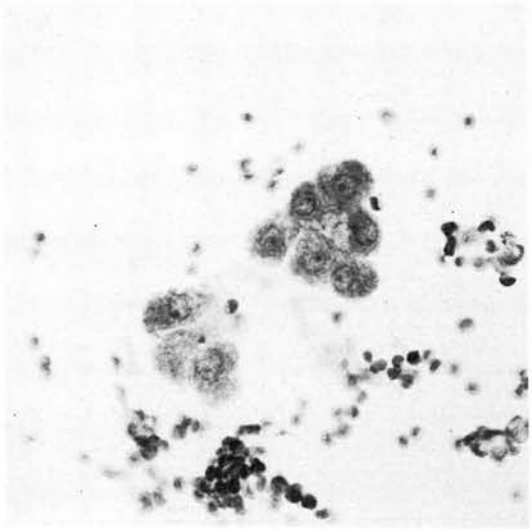


Fig. 3. *Cytological preparation of a cyst aspirate stained to show the specific glycolipid granules found in apocrine cells. The vesicular nuclei with prominent nucleoli, also a feature of apocrine epithelium, are easily seen (PAS diastase × 480).*

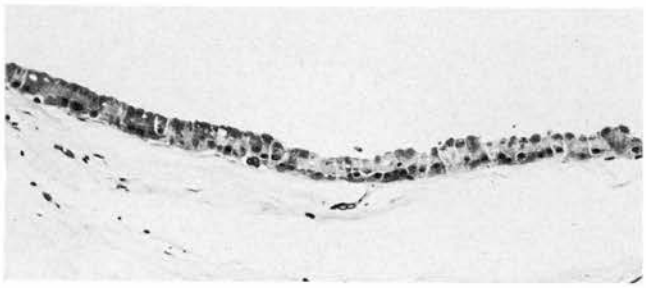


Fig. 4. *Dissection specimen of an apocrine cyst. The cells are columnar and have basally situated nuclei, abundant cytoplasm and apical snouts containing deeply staining intracytoplasmic glycolipid granules (H & E × 128).*

Fig. 5. *Dissection specimen of a flattened epithelial cyst. The epithelium is attenuated and flattened and few cells are visible in any one section (H & E × 256).*

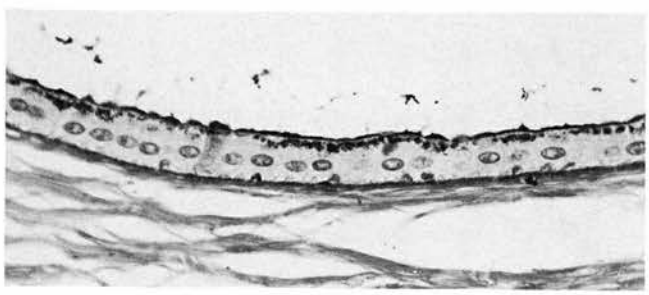


Fig. 6. *Dissection specimen showing the apical position of the glycolipid granules in apocrine epithelium (PAS diastase × 256).*

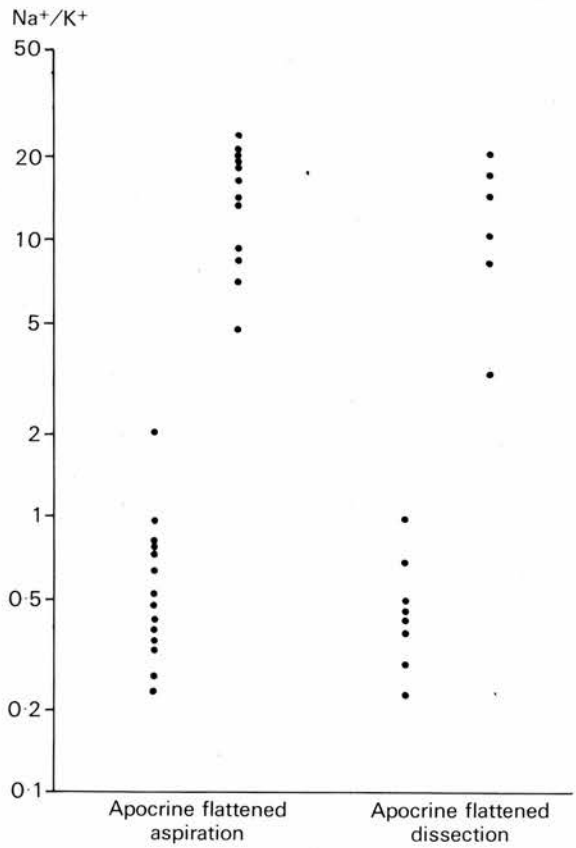


Fig. 7. *The ratios of sodium (Na^+) and potassium (K^+) in the cysts where the epithelium was assessed as apocrine or flattened by examination of aspiration and dissection preparations.*

to distinguish completely between apocrine and flattened epithelial cysts, those cysts lined by apocrine epithelium having a ratio of 2 or less and those lined by simple flattened epithelium a ratio of greater than 3.

Discussion

Approximately 7 per cent of all women in the Western world develop a breast cyst (6). Certain studies have shown that these women are at increased risk of developing breast cancer (5, 6) although others have been unable to confirm this finding (7). In view of their frequency and their possible relationship to breast cancer, it is surprising that little is known of the composition or derivation of human breast cysts. Recently breast cyst fluids have been divided into two major groups on the basis of the relative concentrations of Na^+ and K^+ (1, 2, 8). These two groups of fluids have also been shown to contain significantly different levels of the androgen conjugate DHA sulphate and to contain different molecular forms of IgA (2–4). The present study has demonstrated that the epithelia lining these two groups of cysts are morphologically different. All cysts where the Na^+/K^+ ratio was 2 or less were lined by apocrine epithelium and those with a ratio of greater than 3 were lined by flattened epithelium.

This subdivision on the basis of the Na^+/K^+ ratio is similar to that used by us to discriminate between cysts with high and low DHA sulphate (1, 2). Cysts with a low Na^+/K^+ ratio had high levels of DHA sulphate. Such cysts have been shown in the present study to be lined by apocrine epithelium. It is of note that apocrine secretion from axillary skin has also been shown to contain high concentrations of DHA sulphate (9).

Electron microscopic studies of breast cyst epithelium have shown that apocrine cells contain many mitochrondria and apical secretory granules, in contrast to flattened epithelial cells which contain few organelles (10). Apocrine secretion is performed by expelling the contents of the intracellular secretory granules and is more accurately termed merocrine (5). The

finding of high potassium, the major intracellular cation, in cysts lined by apocrine epithelium, correlates well with what is known of the ultrastructure and method of secretion of epithelium of apocrine type.

Studies of patients with multiple cysts in one or both breasts have shown that, in the majority, the cysts had a similar electrolyte composition (2) and were therefore likely to be lined by the same type of epithelium. This observation suggests there may be a factor which determines whether patients develop cysts lined by apocrine or flattened epithelium. Apocrine change has been reported to occur more frequently in populations at high risk of breast cancer (11–13) and the results from this study show that the presence of apocrine epithelium in cystic disease may be detected by electrolyte analysis of breast cyst fluid. It is therefore possible that such analysis will be helpful in identifying patients at increased risk of breast cancer.

Acknowledgements

Thanks are accorded to Dr T. J. Anderson and Dr J. Lamb of the University Department of Pathology for assessing all cytological and histological preparations and Miss C. Hughes for secretarial assistance. Dr J. M. Dixon is a Wellcome Surgical Research Fellow and acknowledges the support of the Wellcome Trust.

References

1. Dixon J. M., Miller W. R., Scott W. N. et al.: Subpopulations of human breast cysts as defined by sodium, potassium and dehydroepiandrosterone content. *Langenbecks Arch. Chir.* 1982; **357**: 195.
2. Miller W. R., Dixon J. M., Scott W. N. et al.: Classification of human breast cysts according to electrolyte and androgen conjugate composition. *Clin. Oncol.* 1983 (In the press.)
3. Yap P. L., Miller W. R., Roberts M. M. et al.: Studies on the composition of human breast cyst fluid. *Br. J. Cancer* 1982; **46**: 516.
4. Dixon J. M., Miller W. R., Yap P. L. et al.: Further studies on human breast cyst fluid. *Br. J. Surg.* 1983; **70**, (In the press.)
5. Azzopardi J. G.: *Problems in Breast Pathology*. London: Saunders, 1979; 57–91.
6. Haagensen C. D., Bodian C. and Haagensen D. E.: *Breast Carcinoma, Risk and Detection*. London: Saunders, 1981; 55–80.
7. Page D. L., Vander Zwag R., Rogers L. W. et al.: Relation between component parts of fibrocystic disease complex and breast cancer. *J. Natl Cancer Inst.* 1978; **61**: 1055–63.
8. Bradlow H. L., Skidmore F. D., Schwartz M. K. et al.: Cation levels in human breast cyst fluid. *Clin. Oncol.* 1981; **7**: 388–90.
9. Labows J. N., Preti G., Hoelzle E. et al.: Steroid analysis of human apocrine secretion. *Steroids* 1979; **34**: 249–98.
10. Ahmed A.: *Atlas of the Ultrastructure of Human Breast Disease*. Edinburgh: Churchill Livingstone, 1978; 41–8.
11. Haagensen C. D., Bodian C. and Haagensen D. E.: *Breast Carcinoma, Risk and Detection*. London: Saunders, 1981; 83–105.
12. Scheurch C., Rosen P. P., Hirut A. T. et al.: A pathologic study of benign breast disease in Tokyo and New York. *Cancer* 1982; **50**: 1899–903.
13. Wellings S. R., Jensen H. M. and Marcum R. G.: An atlas of subgross pathology of the human breast with reference to possible precancerous lesions. *J. Natl Cancer Inst.* 1975; **55**: 231–73.

Human breast cystic disease

J. M. Dixon, Wellcome Surgical Research Fellow, and W. R. Miller, Lecturer, University Department of Clinical Surgery, Royal Infirmary, Edinburgh, EH3 9YW.

INTRODUCTION

Breast cysts were first distinguished from carcinomas in 1829 by Astley Cooper[1]. They are the commonest cause of a benign breast mass affecting approximately 7 per cent of all women in the Western World[2]. It has been reported that these women may be at increased risk of subsequent breast cancer[2,3]. In view of the length of time which has passed since their identification, their frequency and association with hyperplasia and breast cancer, it is surprising that comparatively little is known of the content and derivation of human breast cysts.

Composition

Recently electrolytes[4,5] and hormones[6,7] in cyst fluids have been measured and shown to vary widely between individual cysts. We have measured sodium (Na^+), potassium (K^+) and the androgen conjugate dehydroepiandrosterone sulphate (DHA sulphate) in 100 cyst fluids and confirmed this wide variation. (Figures 1 and 2). However, we found significant interrelationships between these three substances, with inverse correlations between Na^+ and both K^+ and DHA sulphate and a direct correlation between K^+ and DHA sulphate (Figures 3a, b and c). It was also evident that values for Na^+ and K^+ were not normally distributed around the mean, indicating that there may be more than one population of cyst fluids. This was particularly evident in a plot of the ratio of Na^+ to K^+ (Figure 4). Cyst fluids could be divided into two groups, one having a high and the other a low Na^+/K^+ ratio. DHA sulphate concentrations in these two populations were significantly different, levels being higher in fluids with low Na^+/K^+ ratios (Figure 5).

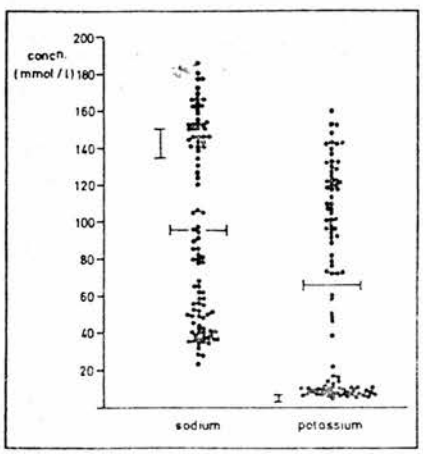


Figure 1. Concentrations of sodium and potassium in human breast cyst fluids. Horizontal lines represent mean values. Vertical lines represent reference range for plasma.

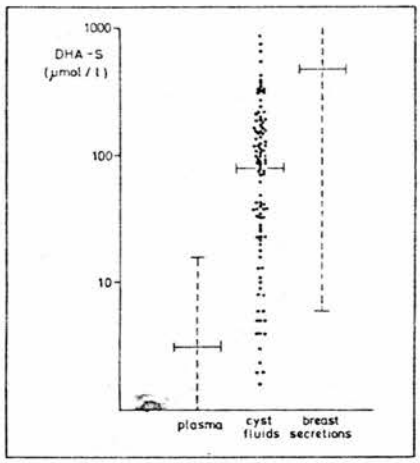


Figure 2. DHA sulphate levels in human breast cyst fluids. Dotted vertical lines represent range in human plasma and breast secretions obtained by nipple aspiration. Horizontal lines represent median values.

Further studies have shown that pH, concentration of albumin and IgG and type of IgA also vary in the two populations of cyst fluids as defined by electrolyte content.

Relationship of epithelial lining of cysts and cyst fluid composition

Morphological studies of the epithelium lining breast cysts have been undertaken to determine whether differences in the composition of the two populations of breast cysts are related to the nature of the lining epithelium. The epithelium lining 40 breast cysts was characterised by either histology or cytology. This was then compared with the composition of the cyst fluid.

Two types of epithelium were identified in both histological and cytological preparations:-

(1) acidophilic cells on haematoxylin and eosin stained sections (H & E), with copious cytoplasm containing granules which stain by the periodic acid Schiff (PAS) technique after diastase digestion, have luminal snouts and nuclei with prominent nucleoli — APOCRINE epithelium.

(2) basophilic cells (on H and E) having less cytoplasm with no specific features — FLATTENED epithelium.

Figures 6, 7, 9 and 10 show examples of apocrine and flattened cells in histological and cytological preparations with the PAS positive glycolipid granules characteristic of apocrine epithelium being demonstrated in Figures 8 and 11.

Comparison of the epithelial lining of the cyst as assessed in histological

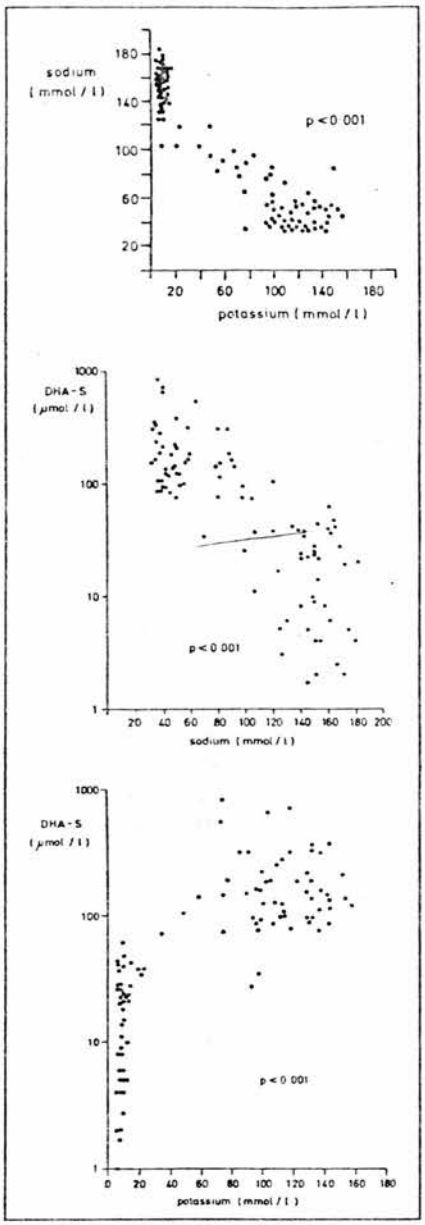


Figure 3. Relationships in human breast cyst fluid between (a) Na^+ and K^+ (b) DHA sulphate and Na^+ (c) DHA sulphate and K^+. Significance values from Kendal rank test.

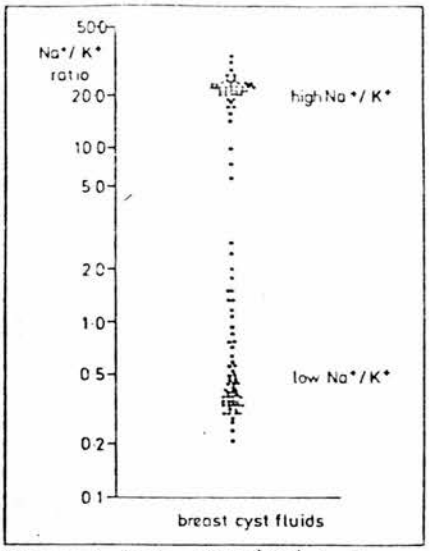


Figure 4. Ratio of Na^+/K^+ in human breast cyst fluids. Two populations are evident, one with a high and the other with a low Na^+/K^+ ratio.

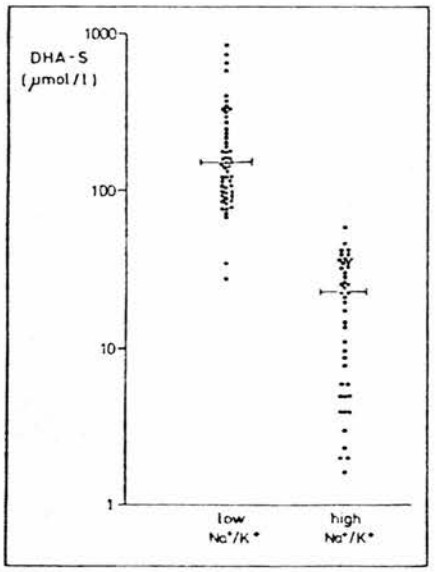


Figure 5. DHA sulphate in human breast cyst fluids subdivided according to electrolyte classification into fluids with high Na^+/K^+ and low Na^+/K^+ ratio.

and aspiration specimens and the Na^+/K^+ ratio in cyst fluid (Figure 6) showed all cysts lined by apocrine epithelium had a lower Na^+/K^+ ratio than those lined by flattened epithelium. Similarly subdivision of cysts according to histology of the epithelial lining split DHA-sulphate values into two separate groups, those with apocrine epithelium having high concentrations and those with flattened epithelium having low concentrations of DHA sulphate (Figure 13).

Apocrine cyst fluids thus have a low Na^+/K^+ ratio and high concentrations of DHA sulphate. Apocrine secretion occurs by expulsion of cellular contents via intracytoplasmic vacuoles[2] and the finding of high potassium concentrations in fluids derived from apocrine

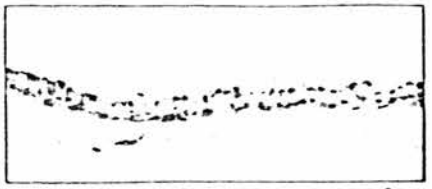


Figure 6. Histological specimen of an apocrine cyst. The cells are columnar, have basally situated nuclei, apical snouts and abundant cytoplasm containing intracytoplasmic glycolipid granules (H & E × 240).

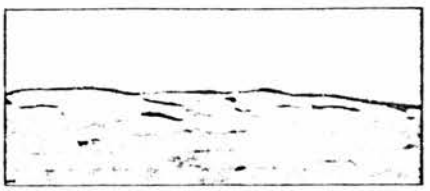


Figure 7. Histological specimen of a flattened epithelial cyst. The epithelium is flat and attenuated and has no specific features (H & E × 480).

Figure 8. Histological specimen showing the apical position of the PAS positive glycolipid granules in apocrine epithelium. (PAS Diastase × 320).

cells is consistent with this activity. It is of interest that apocrine secretion from axillary skin has been shown to contain high concentrations of DHA sulphate[8]. It is also of note that the colour range of apocrine axillary sweat (yellow to green to bluish black) is identical to that seen in breast cysts[9].

Relationship of cyst fluid populations to natural history and breast cancer risk

Whilst two populations of breast cysts may be defined on the basis of composition and the nature of epithelial lining, it remains to determine whether these two subgroups have any clinical relevance. Preliminary studies suggest that these two groups differ in terms of their natural history and their association with the subsequent development of breast cancer.

We know that about half of all women, who present with cystic disease will have a single cyst, a third of patients will develop between 2 and 5 cysts and the remainder have in excess of 5[2]. Our own data from a prospective analysis of 100 consecutive patients followed over 2 years show 45 per cent of patients had 1 cyst, 46 per cent had 2-5 cysts and 9 per cent had in excess of 5. All cysts aspirated from these 100 patients were classified on the basis of electrolyte composition as apocrine ($Na^+/K^+ < 3$) or flattened ($Na^+/K^+ \geq 3$). The relationship of cyst type to the natural history of cystic disease was then examined.

Figure 9. Cytological preparation of a cyst aspirate showing apocrine cells. The cells have copious pale granular cytoplasm with vesicular nuclei showing prominent nucleoli (Papanicolaou × 480).

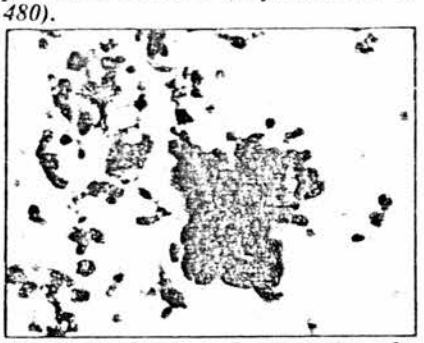


Figure 10. Cytological preparation of a cyst aspirate showing simple flattened epithelium. The cells have a small amount of cytoplasm, deeply staining nuclei and no special features. (Papanicolaou × 480).

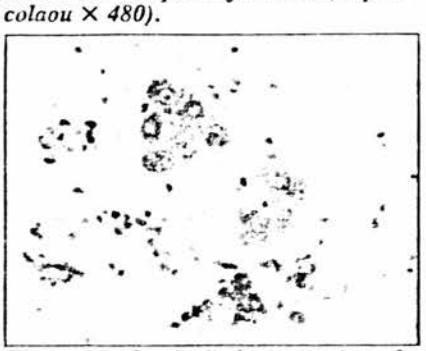


Figure 11. Cytological preparation of a cyst aspirate stained to show the specific glycolipid granules found in apocrine cells. The vesicular nuclei with prominent nucleoli, also a feature of apocrine epithelium are easily seen. (PAS diastase × 480).

Of the 100 patients, 43 developed single or multiple cysts of flattened type, 44 patients developed single or multiple cysts of apocrine type and 13 patients had mixtures of the two types of cysts. Patients with a single cyst were more likely to have a flattened cyst and as the number of cysts aspirated in each patient increased, so the proportion of apocrine cysts also increased. (Table 1).

There were 56 episodes where patients had multiple simultaneous cysts and in 48 (88 per cent) all cysts were of the same type, ie all apocrine or all flattened. A total of 43 patients developed sequential cysts and in 32 (74

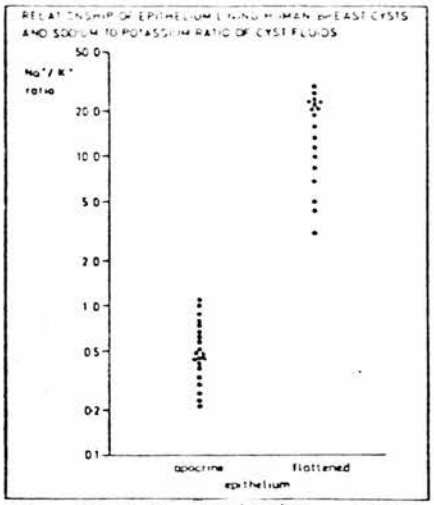


Figure 12. Ratio of Na^+/K^+ in cyst fluids where the lining epithelium of the cyst was assessed as apocrine or flattened by examination of histological or aspiration specimens.

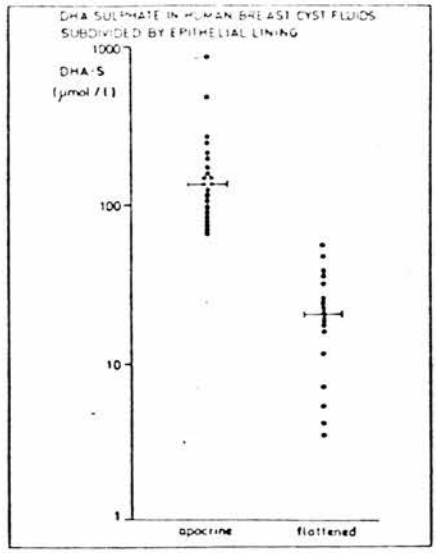


Figure 13. DHA sulphate levels in cyst fluids where the lining epithelium of the cyst was apocrine or flattened.

per cent) of these, all cysts aspirated over the two year period were of the same type. Thus, patients who have multiple cysts, whether they are simultaneous or sequential usually develop cysts of one or other type.

A comparison of the frequency of further cysts in the groups of patients who presented with either apocrine or flattened cysts showed that patients who had single or multiple apocrine cysts were more than 5 times more likely to develop further cysts.

A review of 400 patients with breast cancer identified 10 patients who had a previous history of cyst aspiration where the aspirated cysts could be classified on the basis of cytology or electrolyte composition. Nine of these patients had single or multiple apocrine cysts and one had a single flattened cyst. This ratio is markedly different to that in the general population with cystic disease (see above) and is in keeping with other observations that apocrine change occurs more frequently in populations at risk of breast cancer[10-12].

Conclusion

Two populations of human breast cysts can be identified. They differ in the nature of the lining epithelium and the composition of cyst fluid. Preliminary studies show that the groups are associated with clinical differences in natural history. It is also possible that these two groups also differ in their association with subsequent breast cancer. Larger numbers of patients and further follow up is necessary to confirm this.

Table 1: Comparison of the % of cysts which were classified as flattened or apocrine by electrolyte composition in group of patients who had totals of 1, 2-5 or >5 cysts aspirated over a 2 year period.

Number of cysts aspirated	Number of patients	% of cysts which were Flattened	Apocrine	Ratio Apocrine / Flattened
1	45	76	24	0.3
2-5	46	25	75	3.0
>5	9	6	94	15.7

Acknowledgements

J. M. Dixon is a Wellcome Surgical Research Fellow and acknowledges the support of the Wellcome Trust. Thanks are accorded to Professor A. P. M. Forrest for allowing us to investigate patients under his care.

Figures 1, 2, 3a, b and c are produced by kind permission of *Clinical Oncology*.

Figures 6 and 8-11 are produced by kind permission of the *British Journal of Surgery*.

References

1. Cooper AP. Disease of the Breast Part 1. London. Longmans, 1929.
2. Haagensen CD, Bodian C and Haagensen DE. Breast carcinoma risk and detection. London. Saunders, 1981. 55-80.
3. Azzopardi JG. Problems in breast pathology. London. Saunders, 1979. 57-91.
4. Bradlow HL, Skidmore FD, Schwartz MK, and Fleisher M. Cation levels in human breast cyst fluid. *Clin Oncol* 1981; 7: 388-390.
5. Gatzy JT, Zayloun MP, Gaskins K and Pearlman WH. Electrolytes of breast cyst fluid. *Clin Chem* 1979; 25: 745-748.
6. Bradlow ML, Schwartz MK, Fleisher M, *et al.* Accumulation of hormones in breast cyst fluid. *J Clin Endocrinol Metab* 1979; 49: 778-782.
7. Miller WR, Roberts MM, Creel RJ, Yap PL, Kelly RW and Forrest APM. Androgen conjugates in human breast cyst fluids. *J Natl Cancer Inst* 1982; 69: 1055-1068.
8. Labows JN, Preti G, Hoelzle E, Leyden J and Kligman A. Steroid analysis of human apocrine secretion. *Steroids* 1979; 34: 249-298.
9. Hurley HJ and Shelley WB. The human apocrine sweat gland in health and disease. Springfield USA. Thomas. 1961: 78-96.
10. Scheurch C, Rosen PP, Hirut AT, *et al.* A pathologic study of benign breast diseases in Tokyo and New York. *Cancer* 1982; 50: 1899-1903.
11. Wellings SR, Jensen HM and Marcum RG. An atlas of subgross pathology of the human breast with reference to possible precancerous lesions. *J Natl Cancer Inst* 1975; 55: 231-275.
12. Page DL, Vander-Zwag R, Rogers LW, Williams LT, Walker WF and Hartmann WH. Relation between component parts of fibrocystic disease complex and breast cancer. *J Natl Cancer Inst* 1978; 61: 1055-1063.

Clinical Oncology 1984, **10**, 221–224

pH of human breast cyst fluids

J. M. Dixon, W. R. Miller and W. N. Scott

University Department of Clinical Surgery, Royal Infirmary, Edinburgh EM3 9YW

(*Accepted for publication 4 June 1984*)

The pH of 106 human breast cyst fluids has been measured immediately following aspiration. Values ranged from pH 6·3 to 7·8. They correlated with the ratio of Na^+ to K^+ in cyst fluid and differed significantly in the two populations of fluids which can be defined on electrolyte composition. Storage of cyst fluid for one month at −20°C resulted in an increase in pH by a mean of 0·9 of a unit. pH provides a simple method for typing cyst fluids and this may be of clinical importance as there is evidence that there are differences in the natural history and association with breast cancer in the two populations of breast cysts.

Introduction

Two separate populations of human breast cysts can be defined on the basis of the ratios of sodium (Na^+) to potassium (K^+) in cyst fluid (Bradlow *et al.*, 1981; Dixon *et al.*, 1982; Miller *et al.*, 1983). It has been shown that this variation in fluid composition reflects different types of epithelium lining the two groups of cysts (Dixon *et al.*, 1983*a*).

The aims of the present study were to measure the pH of fresh cyst fluid and to determine if differences in pH exist between the two populations of cyst fluids defined on electrolyte composition and to investigate the effect of storage on pH.

Patients, materials and methods

One hundred and six breast cyst fluids were aspirated from 74 patients into air tight syringes, placed on ice and pH measured within one hour, using a Corning 178 pH/blood gas analyser. Na^+ and K^+ concentrations were measured in the same fluids by flame photometry (EEL model 150 flame photometer) after dilution 1 in 200 and 1 in 400 with distilled water. Fluids were then stored at −20°C and pH was remeasured in 10 samples after 1 week and 1 month of storage.

Statistical correlation of pH and Na^+/K^+ ratio was by the Kendall rank test. Comparison of pH in the two populations of cysts was made using the Wilcoxon rank sum test.

0305–7399/84/030221+04 $03.00/0

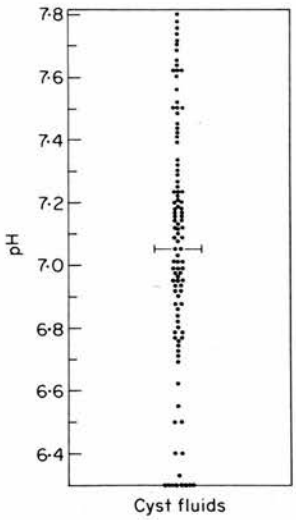


Figure 1. pH of 106 breast cyst fluids measured within 1 hour of aspiration. Horizontal bar represents mean value.

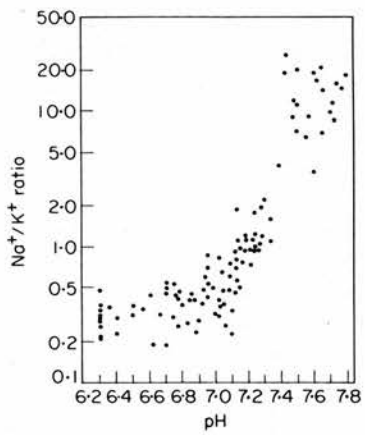


Figure 2. Correlation of pH and the Na^+/K^+ ratio of 106 breast cyst fluid.

Results

The pH values of the 106 cyst fluids ranged from 6·3 to 7·8 (Figure 1). There was significant positive correlation between pH and Na^+/K^+ ratio, $p < 0{\cdot}001$ by the Kendal rank test (Figure 2). Composition of pH in fluids subdivided into different types according to Na^+/K^+ ratio showed no overlap in values between the two populations (Figure 3), the difference between the groups being significant by the Wilcoxon rank sum test ($p < 0{\cdot}001$).

The effect of storage on pH in 10 cyst fluids is shown in Figure 4. After 1 week pH had increased in all fluids by a mean of 0·4 and by 1 month it had increased further by a mean of 0·9 of a pH unit over initial values.

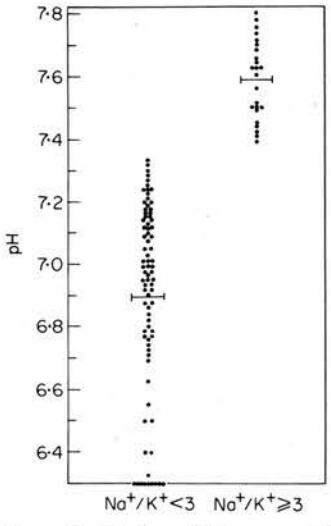


Figure 3. pH of cyst fluids separated into two populations on the basis of Na^+/K^+ ratio. The horizontal bars represents the mean values of the two groups of fluids.

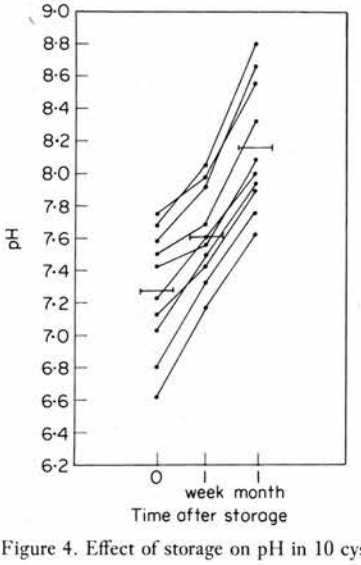


Figure 4. Effect of storage on pH in 10 cyst fluids.

Discussion

Human breast cyst fluids have been previously reported to be alkaline, with a pH range of 7·6–9·0 (Gatsy *et al.*, 1979). These measurements were however performed on stored samples and this study has shown that fresh cyst fluids are acidic or neutral and only become alkaline on storage. pH has been shown to correlate with the ratio of Na^+ to K^+ in cyst fluid and to differ in the two populations of cyst fluids defined on the basis of Na^+/K^+ ratio. Cysts with a low Na^+/K^+ ratio are thought to be lined by apocrine epithelium and cysts with a high ratio to be lined by flattened epithelium (Dixon *et al.*, 1983*a*). In this study apocrine cysts have been shown to have a lower

pH than flattened cysts. This is of interest as apocrine secretion occurs by expelling the contents of intracellular secretory granules (Azzopardi, 1979) and intracellular fluid has a lower pH than extracellular fluid (Waddell & Bates, 1969). Apocrine secretion from the axilla has also been noted to be acidic (Hurley & Shelley, 1960). In contrast the fluid in flattened cysts is considered to arise by transudation (Dixon *et al.*, 1983*b*). The findings of different pH values in the two groups of cysts thus correlate well with the different modes of formation which have been proposed.

It appears that patients with apocrine cysts are more likely to develop further cysts and may be at a greater risk of breast cancer (Haagensen *et al.*, 1981; Dixon & Miller unpublished). As apocrine cyst fluids are acidic, measurement of pH may be useful in the determination of cyst fluid type. Recently we have used Multistix (Ames Division Miles Laboratories Limited, England) to assess pH immediately after aspiration. This has proved a useful, simple method of identifying cyst fluid type and studies on its value are continuing.

Acknowledgements

J. M. Dixon is a Wellcome Surgical Research Fellow and acknowledges the support of the Wellcome Trust. Thanks are accorded to Professor A. P. M. Forrest for allowing us to study patients under his care and to the Department of Clinical Chemistry, Royal Infirmary, Edinburgh for the use of their Corning pH/Blood gas analyser.

References

Azzopardi, J. G. (1979). In: *Problems in Breast Pathology* pp. 55–91. London: W. B. Saunders.

Bradlow, H. L., Skidmore, F. D., Schwartz, M. K. & Fleisher, M. (1981). Cation levels in human breast fluid. *Clinical Oncology* **7**, 388–90.

Dixon, J. M., Miller, W. R., Scott, W. N., Steele, R. J. C. & Forrest, A. P. M. (1982). Subpopulations of human breast cysts as defined by sodium, potassium and dehydroepiandrosterone sulphate content. *Langenbecks Archiv. Fur Chirugie* **357**, 195.

Dixon, J. M., Miller, W. R., Scott, W. N. & Forrest, A. P. M. (1983*a*). The morphological basis of human breast cyst populations *British Journal of Surgery* **70**, 604–6.

Dixon, J. M., Miller, W. R., Yap, P. L., Scott, W. N. & Forrest, A. P. M. (1983*b*). Further studies on human breast cyst fluids. *British Journal of Surgery* **70**, 684.

Gatsy, J. T., Zayloun, M. P., Gaskins, K. & Pearlman, W. H. (1979). Electrolytes of breast cyst fluid. *Clinical Chemistry* **25**, 745–8.

Haagensen, C. D., Bodian, C. & Haagensen, D. E. (1981). In: *Breast Carcinoma: Risk and Detection.* pp. 55–79, 83–105. Philadelphia: W. B. Saunders.

Hurley, H. J. & Shelley, W. B. (1961). In: *The Human Apocrine Sweat Gland in Health and Disease.* pp. 78–96. Springfield: C. C. Thomas.

Miller, W. R., Dixon, J. M., Scott, W. N. & Forrest, A. P. M. (1983). Classification of human breast cysts according to electrolyte and androgen conjugate composition. *Clinical Oncology* **9**, 227–32.

Waddel, W. J. & Bates, R. G. (1969). Intracellular pH. *Physiology Reviews* **49**, 285–329.